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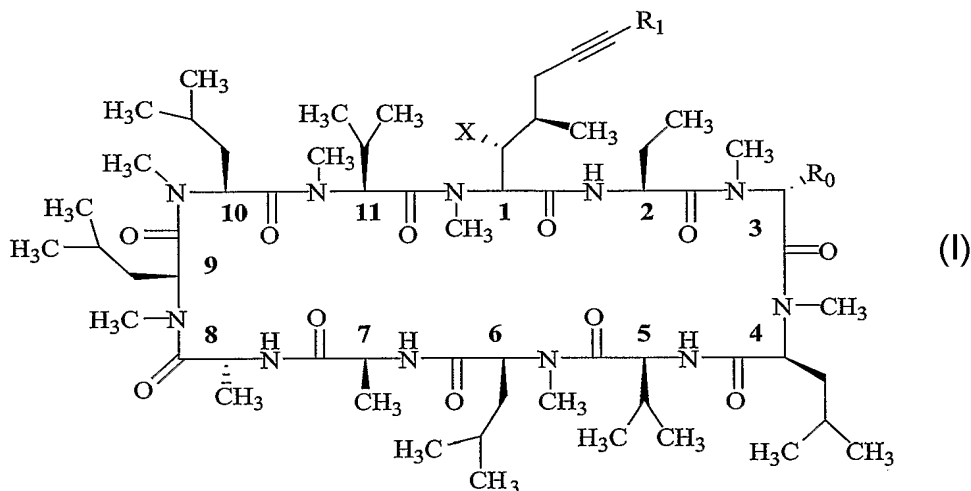
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(54) Title: NOVEL CYCLOSPORIN ALKYNES AND THEIR UTILITY AS PHARMACEUTICAL AGENTS



(57) Abstract: The compounds of the present invention are represented by the chemical structure found in Formula (I), Formula (I), or a pharmaceutically acceptable salt thereof, with X, R<sub>0</sub>, and R<sub>1</sub> defined herein.

## NOVEL CYCLOSPORIN ALKYNES AND THEIR UTILITY AS PHARMACEUTICAL AGENTS

[0001] This application claims the benefit of U.S. Provisional Patent  
5 Application Serial No. 60/616,591, filed October 6, 2004, which is hereby  
incorporated by reference in its entirety.

### FIELD OF THE INVENTION

[0002] The present invention discloses novel cyclosporin alkynes and their  
10 utility as immunosuppressive agents and pharmaceutical agents for treatment of other  
diseases. Methods for preparation of such compounds are also disclosed.

### BACKGROUND OF THE INVENTION

[0003] Cyclosporin A (CsA), a neutral cyclic undecapeptide isolated from the  
15 fungus *Tolypocladium inflatum* and currently marketed as Neoral<sup>®</sup> and Sandimmune<sup>®</sup>  
(Novartis, Basel, Switzerland), has been widely used for the prevention of organ  
transplant rejection. Cyclosporin A exerts its immunosuppressive effect at the  
molecular level by binding to the cyclophilin peptidylprolyl cis/trans isomerase. The  
resulting complex of cyclosporin A and cyclophilin binds to calcineurin, a  
20 Ca<sup>2+</sup>/calmodulin-dependent phosphatase, and inhibits its phosphatase activity.  
Calcineurin regulates the translocation of the transcription factor nuclear factor of  
activated T-cell (NFAT) and the subsequent expression of early genes necessary for  
T-cell proliferation. Inhibition of the phosphatase activity of calcineurin by the  
cyclosporin A-cyclophilin complex prevents NFAT nuclear localization and  
25 suppresses the expression of genes like IL-2, ultimately leading to  
immunosuppression (Matsuda et al., "Mechanisms of Action of Cyclosporin,"  
*Immunopharmacology*, 47:119-125 (2000)).

[0004] Cyclosporin A also has potential therapeutic application in the  
treatment of autoimmune diseases such as rheumatoid arthritis, Crohn's disease,  
30 psoriasis, and chronic inflammatory diseases, such as asthma. Unfortunately, clinical  
utility for these indications has not been realized because of the side effects such as  
nephrotoxicity, hypertension, hepatotoxicity, anaemia, and gastrointestinal intolerance

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that occur with clinically effective doses of cyclosporin A. The toxicity associated with cyclosporin A is believed by many experts working in the immunosuppression therapeutic area to be mechanism based (Lazarova et al., "Cyclosporin A Analogues: Recent Advances," *Expert Opin. Ther. Patents*, 13:1327-1332 (2003)). Indeed, 5 the goal of discovering novel cyclosporin analogues with improved therapeutic index has yet to be achieved despite the significant efforts in this drug discovery area over the last three decades (U.S. Patent No. 5,525,590 to Bollinger et al.; U.S. Patent No. 5,643,870 to Boelsterli et al.; U.S. Patent No. 5,639,852 to Rich et al.; U.S. Patent No. 5,236,899 to Durette,; U.S. Patent No. 5,122,511 to Patchett et al.; U.S. Patent 10 No. 4,914,188 to Dumont et al.; U.S. Patent No. 4,771,122 to Seebach; U.S. Patent No. 4,764,503 to Wenger; U.S. Patent No. 4,396,542 to Wenger; U.S. Patent No. 4,210,581 to Ruegger et al.).

[0005] More recent efforts to find novel cyclosporin analogues with potent immunosuppressive activity and decreased toxicity are underway and have led to 15 compounds such as ISA<sub>TX</sub>247. Preclinical observations indicate that ISA<sub>TX</sub>247 has the potential to be significantly more potent and less toxic than other immunosuppressants in its class already available on the market for the prevention of transplant rejection. ISA<sub>TX</sub>247 is in phase II clinical trials for the prevention of organ rejection after transplantation and for the treatment of psoriasis (Abel et al., 20 "ISA<sub>TX</sub>247: A Novel Calcineurin Inhibitor," *J. Heart Lung Transplant*, 20:161 (2001); Aspeslet et al., "ISA<sub>TX</sub>247: A Novel Calcineurin Inhibitor," *Transplantation Proceedings*, 33:1048-1051 (2001); U.S. Patent Nos. 6,605,593 and 6,613,739 to Naicker et al.).

[0006] A "soft" drug approach (Lazarova et al., "Synthesis and Biological 25 Evaluation of Cyclosporin A Analogues: Potential Soft Drugs for the Treatment of Autoimmune Diseases," *J. Med. Chem.*, 46:674-676 (2003)) has also recently been described that seeks to minimize the toxicity of immunosuppressive cyclosporin A derivatives used for the treatment of autoimmune diseases (PCT International Patent Publication No. WO 03/033010 to Or et al.) and respiratory diseases, such as asthma 30 (PCT International Patent Publication No. WO 02/069902 to Or et al.).

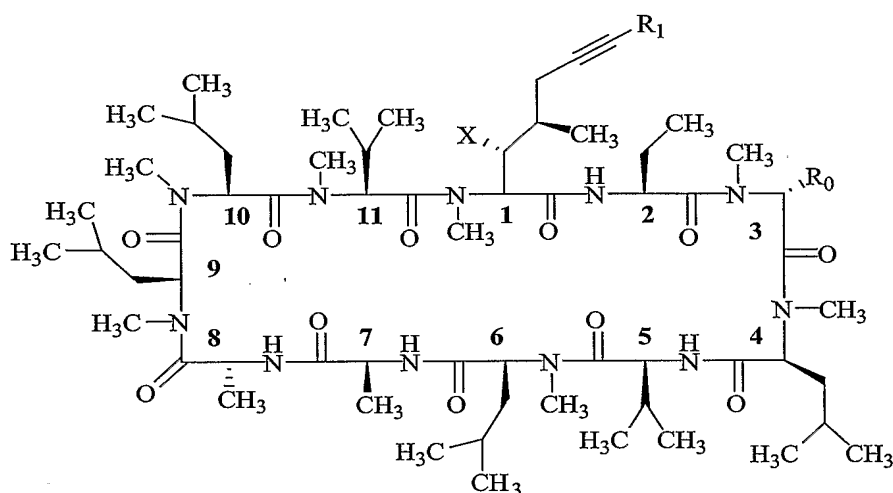
[0007] There is still a large need for novel cyclosporin analogues with improved therapeutic index.

[0008] The present invention is directed to achieving these objectives.

## SUMMARY OF THE INVENTION

[0009] The compounds of the present invention are represented by Formula I,

5 as shown below:



### Formula I

10

where:

X is OH or OAc;

15  $R_0$  is H,  $CH_2OH$ , or  $CH_2OR_2$ ;

$R_1$  is selected from the group consisting of:

hydrogen;

halogen;

20 C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain;

C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain containing substitution or substitutions selected from the group consisting of deuterium, halogen, nitrogen, sulfur, and silicon atom or atoms;

25 C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain containing a function group or function groups selected from the group consisting of alcohol, ether, aldehyde, ketone, carboxylic ester, and amide;

C<sub>2</sub>-C<sub>4</sub> saturated or unsaturated, straight or branched carbon chain containing an aryl or a heteroaryl;

30 C<sub>3</sub>-C<sub>6</sub>-substituted and unsubstituted cycloalkyl;  
substituted and unsubstituted aryl;

substituted and unsubstituted heteroaryl;

$$-\text{CH}_2\text{OH};$$

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-CHO;  
-CH=N-OR<sub>3</sub>; and  
-CH=N-NR<sub>3</sub>R<sub>4</sub>;

- 5 R<sub>2</sub> is selected from the group consisting of:  
alkanoyl;  
alkenoyl;  
alkynoyl;  
aryloyl;  
10 arylalkanoyl;  
alkylaminocarbonyl;  
arylaminocarbonyl;  
arylalkylaminocarbonyl;  
alkyloxycarbonyl;  
15 aryloxycarbonyl; and  
arylalkyloxycarbonyl;

- R<sub>3</sub> or R<sub>4</sub> are the same or different and independently selected from the group consisting of:  
20 hydrogen;  
C<sub>1</sub>-C<sub>6</sub> saturated straight or branched carbon chain;  
C<sub>3</sub>-C<sub>6</sub> unsaturated straight or branched carbon chain;  
C<sub>3</sub>-C<sub>6</sub>-substituted and unsubstituted cycloalkyl;  
C<sub>1</sub>-C<sub>4</sub> carbon chain containing an aryl or heteroaryl;  
25 substituted and unsubstituted aryl;  
substituted and unsubstituted heteroaryl;  
alkanoyl;  
alkenoyl;  
alkynoyl;  
30 aryloyl;  
arylalkanoyl;  
alkylaminocarbonyl;  
arylaminocarbonyl;  
arylalkylaminocarbonyl;  
35 alkyloxycarbonyl;  
aryloxycarbonyl; and  
arylalkyloxycarbonyl; and

- 40 R<sub>3</sub> together with R<sub>4</sub> results in the formation of a cyclic moiety of C<sub>2</sub>-C<sub>6</sub> optionally containing heteroatom or heteroatoms,

or a pharmaceutically acceptable salt thereof.

- [0010] The present invention discloses chemically modified cyclosporin analogues containing a carbon-carbon triple bond on the side chain of the position one  
45 amino acid and optionally a substitution on the position three amino acid of cyclosporin A. In particular, the present invention discloses novel cyclosporin alkyne

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analogues containing a conjugated system of a carbon-carbon triple bond with an aryl, a carbon-carbon double bond, a carbon-nitrogen double bond, or another carbon-carbon triple bond.

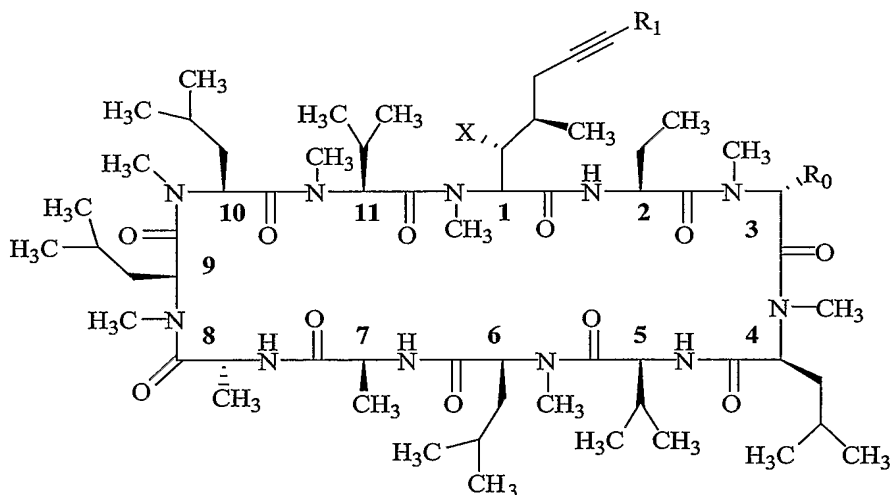
[0011] The present invention provides novel cyclosporin analogues which are effective as immunosuppressive agents. Some compounds of the present invention possess enhanced immunosuppressive activity over cyclosporin A. These compounds also possess utility in the treatment of ocular allergy and dry eye, as well as autoimmune and chronic inflammatory diseases, such as asthma, rheumatoid arthritis, multiple sclerosis, psoriasis, and ulcerative colitis.

### BRIEF DESCRIPTION OF THE DRAWING

[0012] Figure 1 depicts the results from a concanavalin A (ConA)-stimulated murine splenocyte assay, where the novel cyclosporin analogue compounds of the present invention (disclosed in Examples 25 and 10) are shown to possess enhanced or similar potency in immunosuppression, compared to cyclosporin A.

### DETAILED DESCRIPTION OF THE INVENTION

[0013] The compounds of the present invention are represented by Formula I, as shown below:



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**Formula I**

where:

5 X is OH or OAc;

R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OR<sub>2</sub>;R<sub>1</sub> is selected from the group consisting of:

- 10 hydrogen;  
halogen;  
C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain;  
C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain containing  
substitution or substitutions selected from the group consisting of deuterium, halogen,  
15 nitrogen, sulfur, and silicon atom or atoms;  
C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain containing a  
function group or function groups selected from the group consisting of alcohol, ether,  
aldehyde, ketone, carboxylic ester, and amide;  
C<sub>2</sub>-C<sub>4</sub> saturated or unsaturated, straight or branched carbon chain containing  
20 an aryl or a heteroaryl;  
C<sub>3</sub>-C<sub>6</sub>-substituted and unsubstituted cycloalkyl;  
substituted and unsubstituted aryl;  
substituted and unsubstituted heteroaryl;  
-CH<sub>2</sub>OH;  
25 -CHO;  
-CH=N-OR<sub>3</sub>; and  
-CH=N-NR<sub>3</sub>R<sub>4</sub>;

R<sub>2</sub> is selected from the group consisting of:

- 30 alkanoyl;  
alkenoyl;  
alkynoyl;  
aryloyl;  
arylalkanoyl;  
35 alkylaminocarbonyl;  
arylaminocarbonyl;  
arylalkylaminocarbonyl;  
alkyloxycarbonyl;  
aryloxycarbonyl; and  
40 arylalkyloxycarbonyl;

R<sub>3</sub> or R<sub>4</sub> are the same or different and independently selected from the group consisting of:

- 45 hydrogen;  
C<sub>1</sub>-C<sub>6</sub> saturated straight or branched carbon chain;  
C<sub>3</sub>-C<sub>6</sub> unsaturated straight or branched carbon chain;  
C<sub>3</sub>-C<sub>6</sub>-substituted and unsubstituted cycloalkyl;  
C<sub>1</sub>-C<sub>4</sub> carbon chain containing an aryl or heteroaryl;  
substituted and unsubstituted aryl;

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substituted and unsubstituted heteroaryl;  
alkanoyl;  
alkenoyl;  
alkynoyl;  
5 arylolyl;  
arylalkanoyl;  
alkylaminocarbonyl;  
arylaminocarbonyl;  
arylalkylaminocarbonyl;  
10 alkyloxycarbonyl;  
aryloxycarbonyl; and  
arylalkyloxycarbonyl; and

15 R<sub>3</sub> together with R<sub>4</sub> results in the formation of a cyclic moiety of C<sub>2</sub>-C<sub>6</sub> optionally containing heteroatom or heteroatoms,

or a pharmaceutically acceptable salt thereof.

[0014] One embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is H.

20 [0015] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is selected from the group consisting of F, Cl, Br, and I.

[0016] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is  
25 selected from the group consisting of CH=CH<sub>2</sub>, CH=CHCH<sub>3</sub>, CH=CHCH<sub>2</sub>CH<sub>3</sub>, C(CH<sub>3</sub>)=CH<sub>2</sub>, CH=CD<sub>2</sub>, CH=CHCD<sub>3</sub>, and CH=CD<sub>2</sub>CD<sub>3</sub>, and where the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

[0017] Another embodiment of the present invention is the above compound  
30 of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is selected from the group consisting of CH=CHF, CH=CHCl, CH=CHBr, CH=CHI, CH=CF<sub>2</sub>, and CH=CCl<sub>2</sub>, and where the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.

[0018] Another embodiment of the present invention is the above compound  
35 of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is selected from the group consisting of C≡CH, C≡CCH<sub>3</sub>, C≡CCD<sub>3</sub>, C≡CCH<sub>2</sub>CH<sub>3</sub>, C≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, and C≡C-cyclopropyl.



[0019] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is selected from the group consisting of CH<sub>2</sub>C≡CH, CH<sub>2</sub>C≡CCH<sub>3</sub>, CH<sub>2</sub>C≡CCH<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub>CH=CH<sub>2</sub>, CH<sub>2</sub>CH=CHCH<sub>3</sub>, and CH<sub>2</sub>CH=CHCH<sub>2</sub>CH<sub>3</sub> and where the carbon-  
 5 carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers

[0020] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is selected from the group consisting of C≡C-C≡CH, C≡C-C≡CCH<sub>3</sub>, C≡CCH=CH<sub>2</sub>,  
 10 C≡CCH=CHCH<sub>3</sub>, CH=CHC≡CH, CH=CHC≡CCH<sub>3</sub>, CH=CHCH=CH<sub>2</sub>, and CH=CHCH=CHCH<sub>3</sub> and where the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers

[0021] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is  
 15 cyclopropyl.

[0022] Another embodiment of the present invention is the above compound of Formula I, where: X is OH or OAc; R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc; and R<sub>1</sub> is selected from the group consisting of CH<sub>2</sub>OH, -CHO, CH(OH)CH<sub>3</sub>, C(=O)CH<sub>3</sub>, CH=N-OCH<sub>3</sub>, CH=N-OCH<sub>2</sub>CH<sub>3</sub>, CH=N-NHCH<sub>3</sub>, and CH=N-N(CH<sub>3</sub>)<sub>2</sub>.

[0023] Other embodiments of the present invention include the above compound of Formula I, where: X = OH or OAc; R<sub>0</sub> = H; and R<sub>1</sub> is selected from the group consisting of H, C<sub>6</sub>H<sub>5</sub>-, *p*-FC<sub>6</sub>H<sub>4</sub>-, *p*-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>-, 2-thiophenyl, CH<sub>2</sub>Ph, CH<sub>2</sub>CH=CH<sub>2</sub>, CH<sub>2</sub>C≡CH, CH<sub>2</sub>C≡CHCH<sub>3</sub>, CH<sub>2</sub>C≡CHSi(CH<sub>3</sub>)<sub>3</sub>, Br, CH<sub>2</sub>Cl, CH=CH<sub>2</sub>, CH=CHCH<sub>3</sub> (trans), CH=CHCH<sub>3</sub> (cis), CH=CHCl (trans), CH=CHCl (cis),  
 25 CH=CHSi(CH<sub>3</sub>)<sub>3</sub> (trans), C(CH<sub>3</sub>)=CH<sub>2</sub>, CH=CHPh, CH=CHCO<sub>2</sub>Et (cis), CH=C=CH<sub>2</sub>, C≡CH, C≡CCH<sub>3</sub>, C≡CCD<sub>3</sub>, C≡CCH<sub>2</sub>CH<sub>3</sub>, C≡CC<sub>4</sub>H<sub>9</sub>, C≡CSi(CH<sub>3</sub>)<sub>3</sub>, C≡C-3-thiophene, C≡C-Ph, C≡CBr, C≡C-cyclopropyl, C≡C-cyclohexyl, C≡CCH<sub>2</sub>OH, C≡CCH<sub>2</sub>OCH<sub>3</sub>, C≡CCH<sub>2</sub>SCH<sub>2</sub>CH<sub>3</sub>, C≡CCH<sub>2</sub>N(CH<sub>3</sub>)<sub>2</sub>, C≡CCH=CH<sub>2</sub>, C≡CC(CH<sub>3</sub>)=CH<sub>2</sub>, C≡CCH=CHCH<sub>3</sub> (cis), C≡CCH=CHCH<sub>3</sub> (trans), CH=CHC≡CH,  
 30 CH=CHC≡CCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, CH=CHC≡C-cyclopropyl, CH<sub>2</sub>OH, CHO, C=N-OCH<sub>3</sub>, and C=N-N(CH<sub>3</sub>)<sub>2</sub>.

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[0024] Other embodiments of the present invention include the above compound of Formula I, where: X = OH or OAc; R<sub>0</sub> = CH<sub>2</sub>OH or CH<sub>2</sub>OAc; and R<sub>1</sub> is selected from the group consisting of H, CH=CH<sub>2</sub>, CH=CHCH<sub>3</sub> (cis), CH=CHCH<sub>3</sub> (trans), and CH=CHCl (cis).

5 [0025] In particular, the present invention relates to novel cyclosporin analogues containing a carbon-carbon triple bond on the side chain of the position one amino acid and optionally a substitution on the position three amino acid of cyclosporin A. More particularly, the present invention relates to novel cyclosporin alkyne analogues, in which the carbon-carbon triple bond conjugating with an aryl, or  
10 a heteroaryl, or a carbon-carbon double bond, or a carbon-nitrogen double bond, or another carbon-carbon triple bond is incorporated.

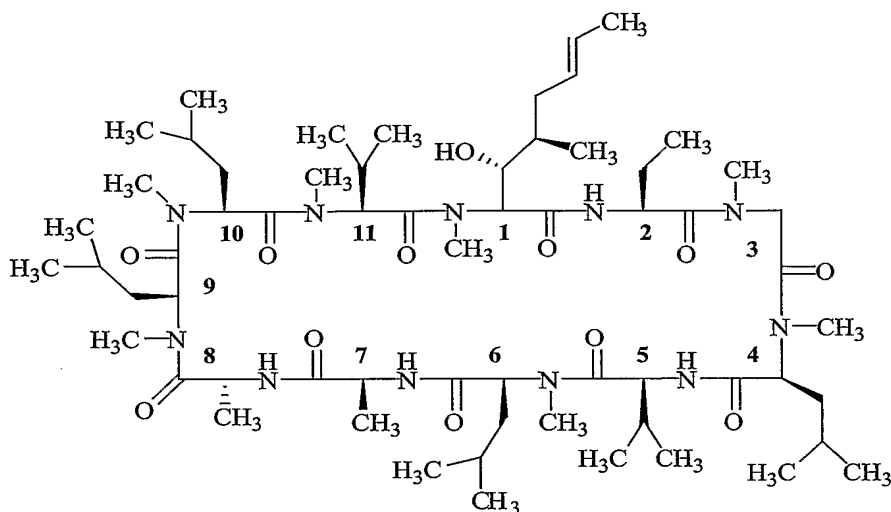
[0026] A carbon-carbon triple bond exists in many natural products (Gung et al., "Total Synthesis of (S)-(-)-(E)-15,16-Dihydrominiquartynoic Acid: A Highly Potent Anticancer Agent," *J. Org. Chem.*, 69:3488-3492 (2004); Ito et al., "Cytotoxic Polyacetylenes from the Twigs of *Ochanostachys amentacea*," *J. Nat. Prod.*, 64:246-  
15 248 (2001), which are hereby incorporated by reference in their entirety). It is well known to use alkynes as pharmaceutical agents. However, only one cyclosporin alkyne, in which a carbon-carbon triple bond replaces the carbon-carbon double bond on the side-chain of the position one amino acid of cyclosporin A, is known in the  
20 literature. Unfortunately, this modification significantly reduces the immunosuppressive activity of cyclosporin A, where this known cyclosporin alkyne shows only 10% relative immunosuppressive activity, compared to cyclosporin A (Rich et al., "Synthesis, Conformation, and Immunosuppressive Activities of Three Analogues of Cyclosporin A Modified in the 1-Position," *J. Med. Chem.*, 33:999-  
25 1009 (1990), which is hereby incorporated by reference in its entirety). In contrast, the novel cyclosporin alkyne analogues of the present invention, which contain a conjugated system of a carbon-carbon triple bond and a carbon-carbon double bond or a carbon-carbon triple bond, possess enhanced immunosuppressive activity over cyclosporin A.

30 [0027] The present invention also discloses methods for preparing compounds represented by Formula I.

[0028] The starting material for the preparation of the compounds of the present invention is cyclosporin A. The structure of cyclosporin A, a

- 10 -

cycloundecapeptide, and the position numbering for each amino acid in the ring is shown below:

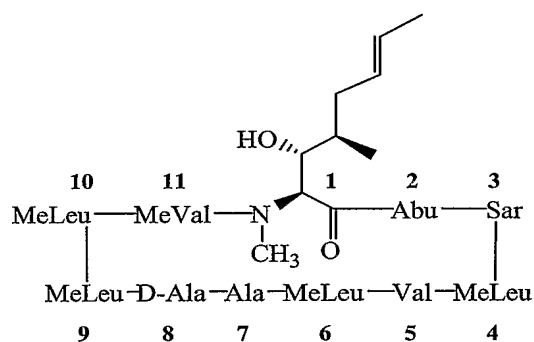


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### Cyclosporin A (CsA)

[0029] Cyclosporin A can also be represented by Formula IIa, as shown below:

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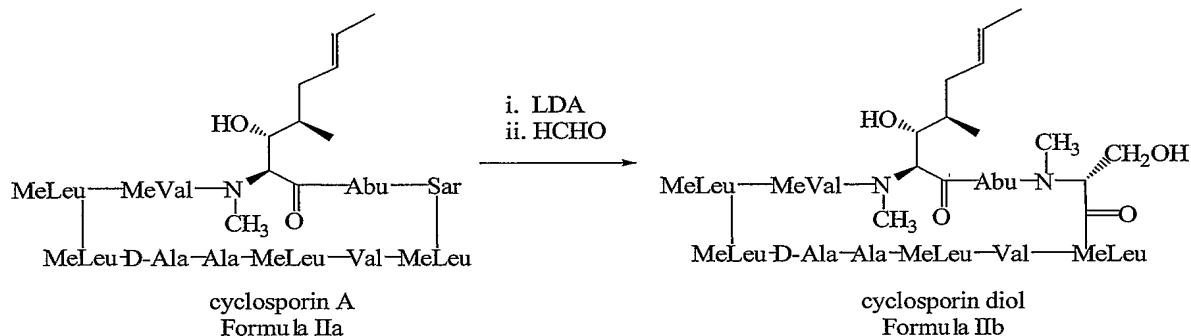
### Formula IIa

15 [0030] The novel cyclosporin analogues of the present invention are derived from cyclosporin A or cyclosporin diol (Formula IIb), a key intermediate prepared by modification on the position three amino acid of cyclosporin A. As shown in Scheme 1, the cyclosporin diol intermediate can be prepared by deprotonation of cyclosporin A with lithium diisopropylamide (LDA) followed by treatment with

formaldehyde (Seebach et al, "Modification of Cyclosporin A: Generation of an Enolate at the Sarcosine Residue and Reaction with Electrophiles," *Helv. Chim. Acta*, 76:1564-1590 (1993), which is hereby incorporated by reference in its entirety).

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Scheme 1



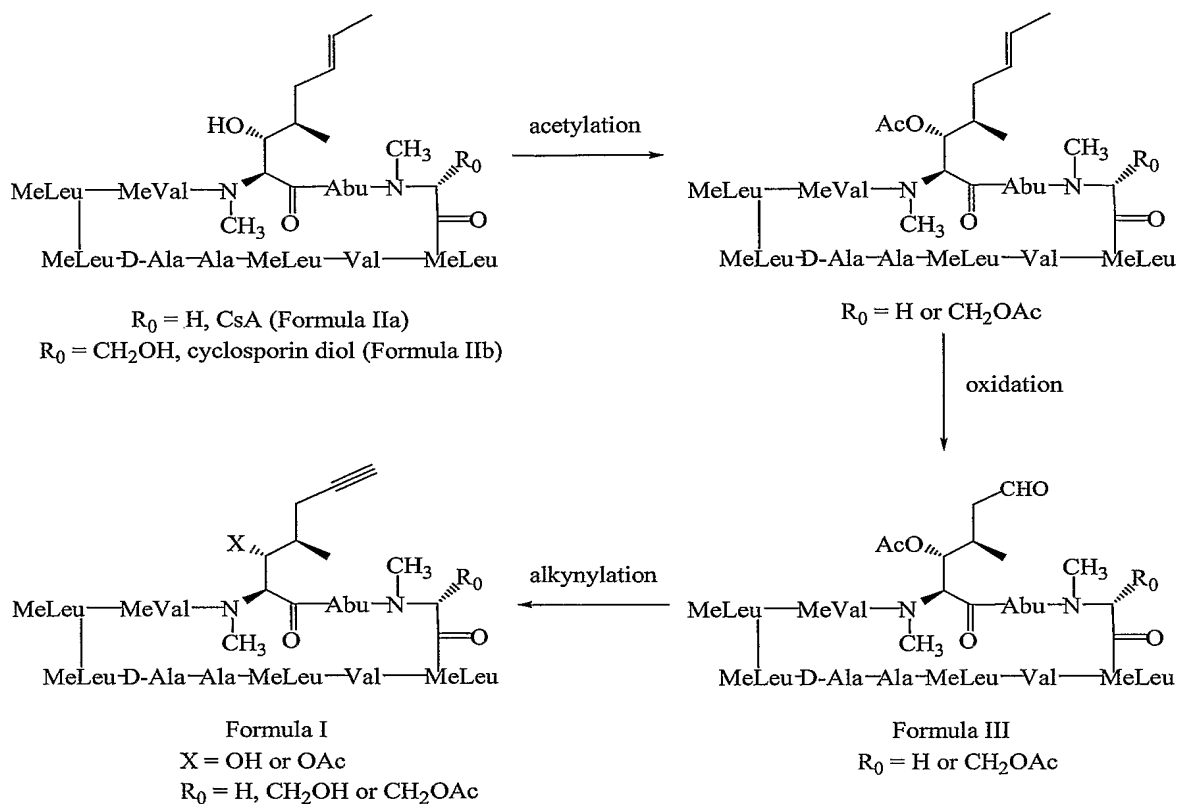
[0031] According to one embodiment of the present invention, novel

10 cyclosporin analogues can be prepared by replacing the carbon-carbon double bond on the side chain of the position one amino acid of cyclosporin A with a carbon-carbon triple bond. As depicted in Scheme 2, acetylation of cyclosporin A (Formula IIa) or the cyclosporin diol intermediate of Formula IIb with acetic anhydride, followed by oxidative cleavage of the double bond with ozone, generates

15 the cyclosporin aldehyde of Formula III smoothly. Treatment of the cyclosporin aldehyde of Formula III with dimethyl (1-diazo-2-oxopropyl)phosphonate in the presence of potassium carbonate in methanol provides cyclosporin alkyne (Formula I, X = OH) in good yield (Müller et al, An Improved One-Pot Procedure for the Synthesis of Alkynes from Aldehydes," *Synlett*, 521-522 (1996), which is hereby

20 incorporated by reference in its entirety). The acetyl protecting group can be removed under these reaction conditions to give the free alcohol directly.

Scheme 2

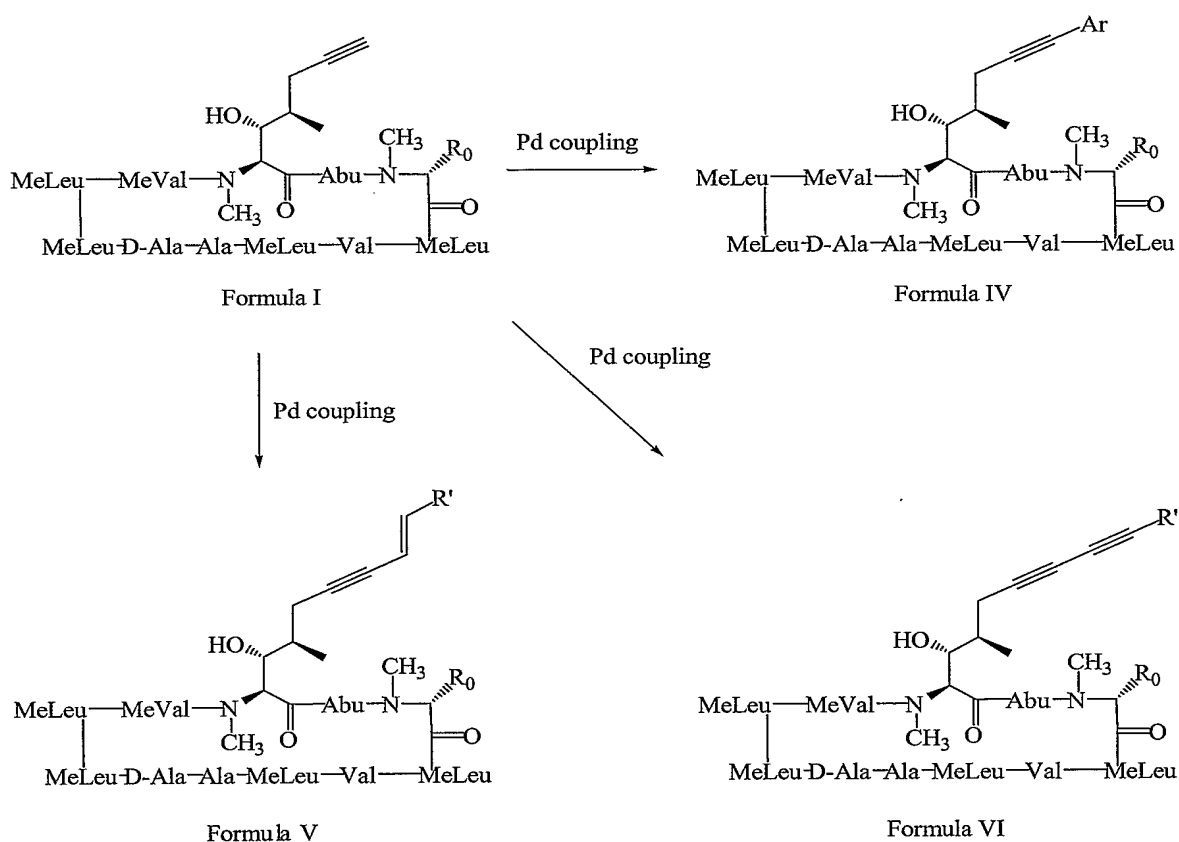


- 5    **[0032]**        The cyclosporin aldehyde of Formula III can also be converted to the cyclosporin alkyne of Formula I ( $\text{X} = \text{OH}$  or  $\text{OAc}$ ) via an alternate method (Scheme 2). Treatment of cyclosporin aldehyde with lithiotrimethylsilyldiazomethane affords the cyclosporin alkyne of Formula I ( $\text{X} = \text{OH}$ ,  $\text{R}_0 = \text{H}$  or  $\text{CH}_2\text{OH}$ ) in good yield (Ohira et al, "Generation of Alkylidenecarbenes by the Alkenation of Carbonyl Compounds with Lithiotrimethylsilyldiazomethane," *J. Chem. Soc. Chem. Commun.*, 721-722 (1992), which is hereby incorporated by reference in its entirety), while the reaction of cyclosporin aldehyde with lithiotrimethylsilyldiazomethane, followed by acidic workup ( $\text{Ac}_2\text{O}$ ), provides the acetyl cyclosporin alkyne of Formula I ( $\text{X} = \text{OAc}$ ,  $\text{R}_0 = \text{H}$  or  $\text{CH}_2\text{OAc}$ ).
- 10
- 15    **[0033]**        Using the above described cyclosporin alkyne (Formula I,  $\text{X} = \text{OH}$ ) as a key intermediate, many novel cyclosporin alkyne derivatives can be prepared via palladium or nickel-mediated couplings. As shown in Scheme 3, Sonogashira coupling of cyclosporin alkyne (Formula I) with various aryl halides, heteroaryl halides, and vinyl halides provides novel cyclosporin arylated alkynes of Formula IV

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and cyclosporin yne-ene analogues of Formula V, respectively. Similarly, the application of palladium-catalyzed coupling to the same key intermediate, cyclosporin alkyne (Formula I), with alkynyl halides leads to the preparation of novel cyclosporin diynes of Formula VI. Utilizing this method, a carbon-carbon triple bond could be introduced step by step to provide a conjugated system of multiple carbon-carbon triple bonds, such as triynes and tetraynes.

Scheme 3



10

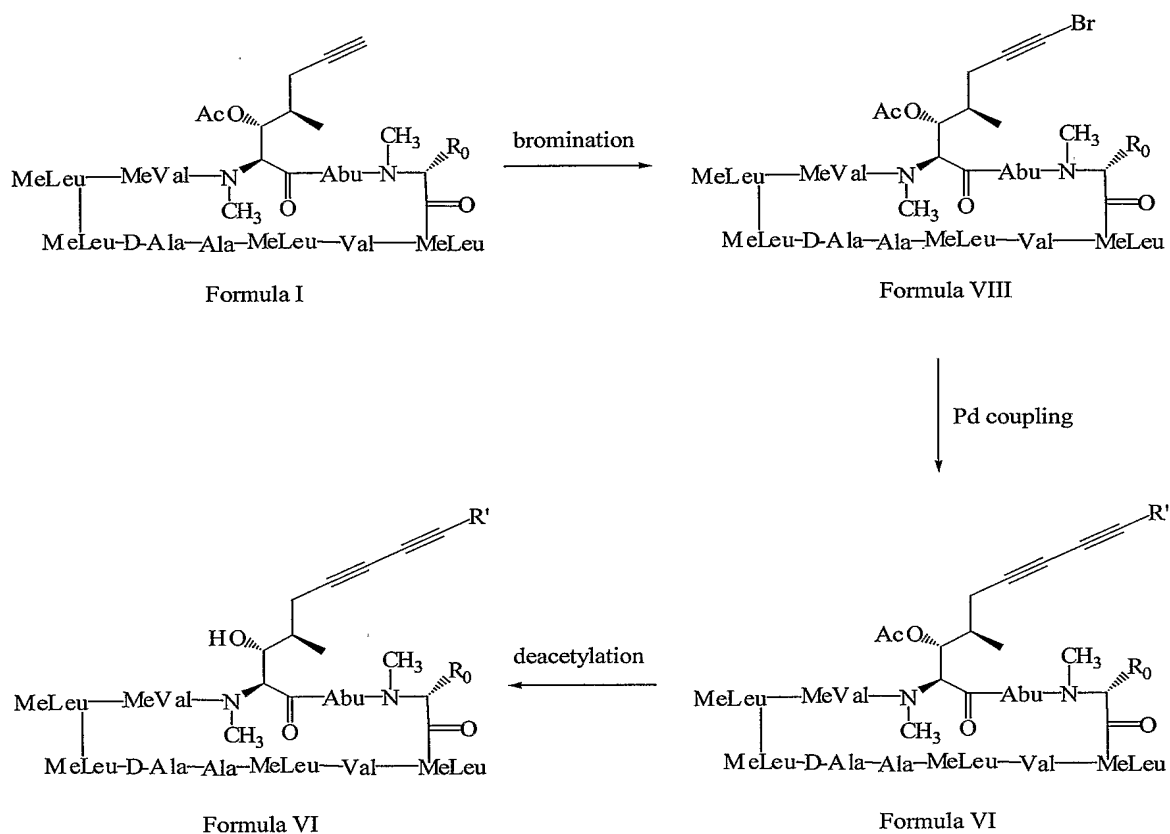
15

**[0034]** As shown in Scheme 4, the cyclosporin diynes of Formula VI can be prepared using an alternative approach. Bromination of cyclosporin alkyne (Formula I, X = OAc, R<sub>1</sub> = H) with *N*-bromosuccinimide in the presence of silver nitrate affords cyclosporin alkynyl bromide (Formula VIII). Using this method, other cyclosporin alkynyl halides, such as cyclosporin alkynyl iodide, can be obtained with *N*-iodosuccinimide instead of *N*-bromosuccinimide. Palladium-catalyzed coupling of

cyclosporin alkynyl bromide (or cyclosporin alkynyl iodide) with various alkynes affords cyclosporin diynes of Formula VI smoothly.

Scheme 4

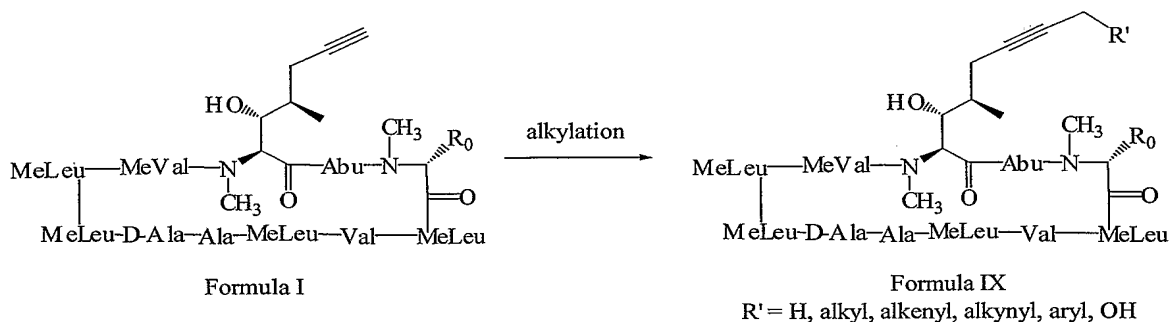
5



[0035] Another embodiment of the present invention relates to the alkylation of cyclosporine alkyne (Formula I, R<sub>1</sub> = H). As shown in Scheme 5, the treatment of cyclosporine alkyne (Formula I, R<sub>1</sub> = H) with alkyl halides or aldehyde in the presence of a base (cesium carbonate, benzyltrimethylammonium hydroxide, or other strong bases) provides the alkylated cyclosporin alkyne (Formula IX).

10

### Scheme 5



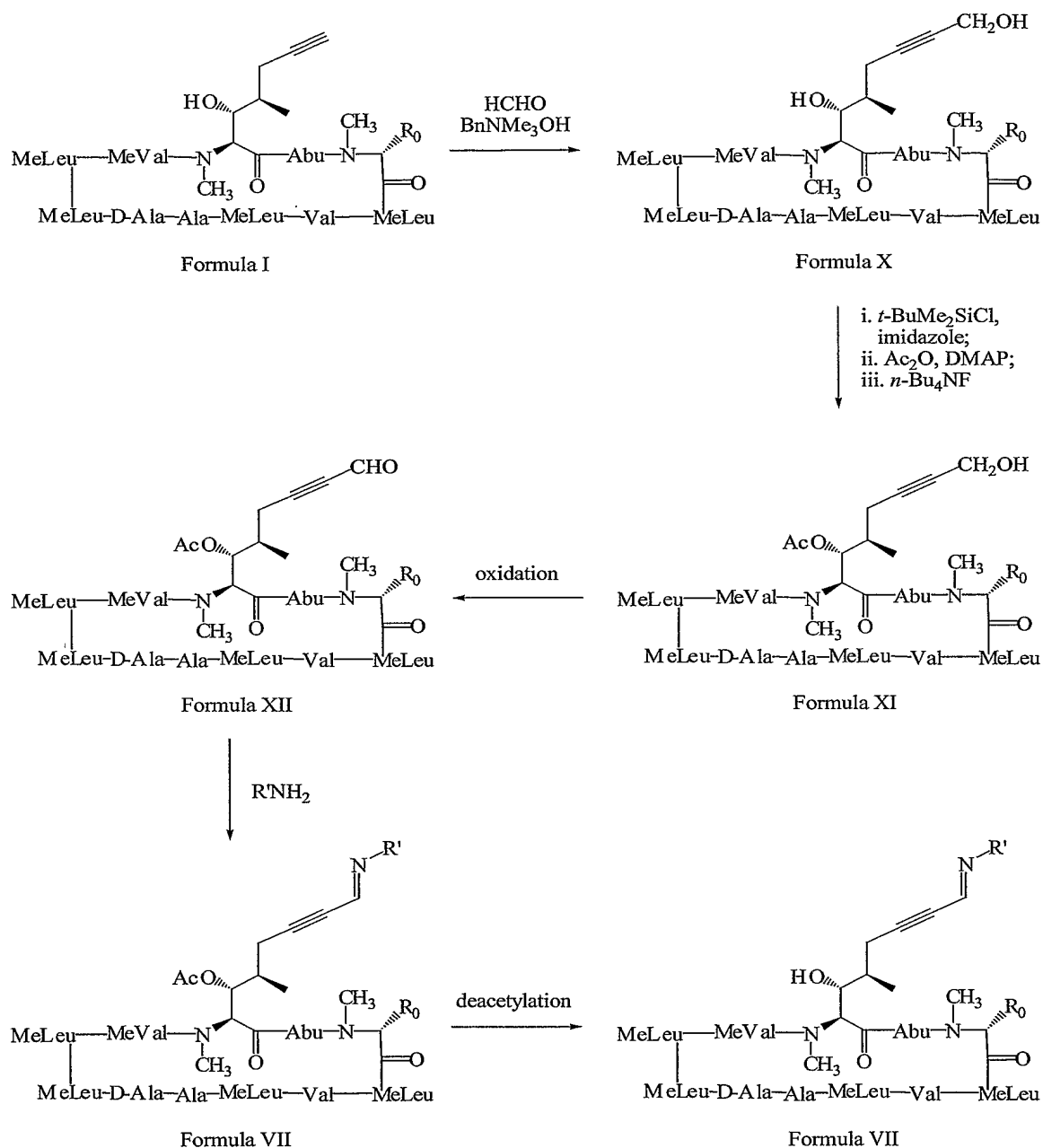
5 [0036] Another embodiment of the present invention relates to the incorporation of a carbon-nitrogen double bond ( $C=N$ ) in the cyclosporin alkyne of Formula I. As shown in Scheme 6, the reaction of cyclosporin alkyne (Formula I,  $R_1 = H$ ) with formaldehyde, using benzyltrimethylammonium hydroxide as a base, provides the cyclosporin diol of Formula X. Selective protection of the primary

10 alcohol of the cyclosporin diol with *tert*-butyldimethylsilyl chloride, followed by acetylation of the second alcohol with acetic anhydride and then desilylation with tetrabutylammonium fluoride, affords the mono-alcohol (Formula XI) smoothly. Swern oxidation of the mono-alcohol affords the cyclosporin aldehyde of Formula XII. Treatment of the aldehyde with hydroxylamine, alkyloxyamines

15 ( $RONH_2$ ), or hydrazines ( $R_2NNH_2$ ) affords the corresponding cyclosporin oximes ( $CH=N-OR$ ) and hydrazones ( $CH=N-NR_2$ ) of Formula VII, respectively.



## Scheme 6



- 5 [0037] The compounds disclosed in the present invention are particularly useful as immunosuppressive agents. Administration of these compounds suppresses the immune response in organ transplant patients and, thus, prevents allograft rejection. The compounds of the present invention possess enhanced or similar immunosuppressive activity, compared to cyclosporin A. For example, as shown in
- 10 Figure 1, the cyclosporin alkyne analogue compound disclosed in Example 25

demonstrates immunosuppressive activity two times more potent over cyclosporin A, while the cyclosporin alkyne analogue compound disclosed in Example 10 shows similar potency to cyclosporin A in the concanavalin A (ConA) stimulated murine splenocyte assay. Table 1 shows the immunosuppressive activities of several novel cyclosporin alkyne analogue compounds disclosed in the present application. (The third column in Table 1 contains cyclosporin A positive control values included for comparison.)

Table 1. Immunosuppressive Activities of Novel Cyclosporin Alkyne Analogue Compounds of the Present Invention

Example Where the Novel Cyclosporin Alkyne Analogue Compound is Disclosed	IC <sub>50</sub> (ng/mL)	IC <sub>50</sub> (ng/mL) of CsA
Example 7	25	15
Example 10	25	20
Example 13	42	15
Example 14	32	15
Example 22	15	18
Example 25	12	20
Example 27	12	31
Example 29	20	31
Example 33	43	15
Example 35	77	18
Example 38	24	18
Example 43	46	18

**[0038]** The compounds disclosed in the present invention also possess utility in the treatment of autoimmune and chronic inflammatory diseases, such as asthma, rheumatoid arthritis, multiple sclerosis, psoriasis, and ulcerative colitis, to name only a few.

**[0039]** In addition, the compounds disclosed in the present invention are useful for the treatment of ocular allergy and dry eye. Allergan is currently marketing a topical formulation of cyclosporin A called Restasis™ (cyclosporin ophthalmic emulsion) for the treatment of keratoconjunctivitis sicca or chronic dry eye syndrome in patients whose tear production is presumed to be suppressed due to ocular inflammation. While the exact mechanism of Restasis™ is unknown, it is thought to act as an immunomodulator with anti-inflammatory effects (“Annual Update 2003:

Ophthalmic Drugs” *Drugs of the Future*, 28(3): 287-307 (2003); Clark et al., “Ophthalmic Drug Discovery,” *Nature Reviews in Drug Discovery*, 2:448-459 (2003), which are hereby incorporated by reference in their entirety).

**[0040]** For treatment of the above mentioned diseases, therapeutically effective doses of the compounds of the present invention may be administered orally, topically, parenterally, by inhalation spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral, as used herein, includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

10 **[0041]** The pharmaceutical compositions containing the active ingredient may be in the form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. The pharmaceutical compositions of the present invention contain the active ingredient formulated with one or more pharmaceutically acceptable carriers. As used herein, the term “pharmaceutical acceptable carrier” means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material, or formulation auxiliary of any type. Some examples of pharmaceutically acceptable carriers are sugars such as lactose, glucose, and sucrose; starches such as corn starch or potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; 25 alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; phosphate buffer solutions; non-toxic, compatible lubricants such as sodium lauryl sulfate and magnesium stearate; as well as coloring agents, releasing agents, sweetening, and flavoring and perfuming agents. Preservatives and antioxidants, such as ethyl or n-propyl p-hydroxybenzoate, can also be included in the pharmaceutical compositions.

30 **[0042]** Dosage forms for topical or transdermal administration of compounds disclosed in the present invention include ointments, pastes, creams, lotions, gels, plasters, cataplasms, powders, solutions, sprays, inhalants, or patches. The active

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component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers, as may be required. The ointments, pastes, creams and gels may contain, in addition to an active compound of the present invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

**[0043]** For nasal administration, the compounds disclosed in the present invention can be administered, as suitable, in liquid or powdered form from a nasal applicator. Forms suitable for ophthalmic use will include lotions, tinctures, gels, ointment and ophthalmic inserts, as known in the art. For rectal administration (topical therapy of the colon), the compounds of the present invention may be administered in suppository or enema form, in solution in particular, for Example in vegetable oil or in an oily system for use as a retention enema.

**[0044]** The compounds disclosed in the present invention may be delivered to the lungs by the inhaled route either in nebulizer form or as a dry powder. The advantage of the inhaled route, over the systemic route, in the treatment of asthma and other diseases of airflow obstruction and/or chronic sinusitis, is that patients are exposed to very small quantities of the drug and the compound is delivered directly to the site of action.

**[0045]** Dosages of the compounds of the present invention employed for the treatment of the maladies identified in the present invention will vary depending on the site of treatment, the particular condition to be treated, the severity of the condition, the subject to be treated (who may vary in body weight, age, general health, sex, and other factors) as well as the effect desired.

**[0046]** Dosage levels ranging from about 0.05 mg to about 50 mg per kilogram of body weight per day are useful for the treatment of the conditions or diseases identified in the present invention. This means the amount of the compound disclosed in the present invention that is administered will range from 2.5 mg to about 2.5 gm per patient per day.

**[0047]** The amount of active ingredient that may be combined with the pharmaceutical carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 2.5 mg

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to 2.5 gm of active compound of the present invention compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to 95 percent of the total composition. Dosage unit forms will generally contain between from about 5 mg to about 500 mg of active compound of the present invention. Dosage for topical preparation will, in general be less (one tenth to one hundredth) of the dose required for an oral preparation.

### EXAMPLES

#### 10 **Example 1 – Preparation of Cyclosporin Acetate**

[0048] A solution of cyclosporin A (5.0 g, 4.16 mmol), acetic anhydride (7.80 mL, 83.2 mmol), and DMAP (760 mg, 6.2 mmol) in methylene chloride (40 mL) was stirred overnight at room temperature under N<sub>2</sub> atmosphere. Saturated sodium bicarbonate solution (200 mL) was added to the solution and stirred for an additional 2 h. The mixture was extracted with ether, washed with 1 N HCl, neutralized with saturated sodium bicarbonate solution, washed with brine, dried over sodium sulfate, and concentrated *in vacuo* to afford cyclosporin acetate (4.92 g, 95%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.57 (d, *J* = 9.6 Hz, 1H), 8.04 (d, *J* = 6.9 Hz, 1H), 7.51 (d, *J* = 9.4 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 5.67 (dd, *J* = 11.0, 4.0 Hz, 1H), 5.60–5.44 (m, 2H), 5.39 (dd, *J* = 11.7, 3.7 Hz, 1H), 5.32–5.13 (m, 4H), 5.06–4.93 (m, 2H), 4.85 (t, *J* = 7.2 Hz, 1H), 4.77 (t, *J* = 9.6 Hz, 1H), 4.65 (d, *J* = 13.7 Hz, 1H), 4.41 (t, *J* = 7.0 Hz, 1H), 3.46 (s, 3H), 3.26 (s, 3H), 3.24 (s, 3H), 3.21 (s, 3H), 3.10 (s, 3H), 2.68 (s, 3H), 2.66 (s, 3H), 2.50–2.35 (m, 1H), 2.25–1.80 (m, 6H), 2.08 (s, 3H), 2.01 (s, 3H), 1.75–1.55 (m, 6H), 1.45–0.75 (m, 55H); ESI MS *m/z* 1245 [C<sub>64</sub>H<sub>113</sub>N<sub>11</sub>O<sub>13</sub> + H]<sup>+</sup>.

#### **Example 2 – Preparation of Acetyl Cyclosporin Aldehyde**

30 [0049] Ozone was bubbled into a solution of cyclosporin acetate from Example 1 (3.0 g, 2.4 mmol) in methylene chloride (70 mL) at –78°C until a blue color was developed. The mixture was degassed with nitrogen for a few min and dimethylsulfide (3 mL) was added at –78°C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was concentrated

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in vacuo and the residue was dissolved in ethyl acetate (300 mL), washed with water (2 × 70 mL) and brine (70 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford acetyl cyclosporin aldehyde (2.79 g, 94%) as a white solid. The crude product was carried to the next step without further purification: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 9.60 (d, *J* = 3.5 Hz, 1H), 8.55 (d, *J* = 9.7 Hz, 1H), 7.96 (d, *J* = 6.8 Hz, 1H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 5.67 (dd, *J* = 11.0, 3.8 Hz, 1H), 5.60–5.45 (m, 2H), 5.32 (dd, *J* = 12.1, 3.3 Hz, 1H), 5.24–5.10 (m, 2H), 5.08–4.90 (m, 2H), 4.84 (t, *J* = 7.1 Hz, 1H), 4.73 (t, *J* = 9.6 Hz, 1H), 4.64 (d, *J* = 13.8 Hz, 1H), 4.41 (t, *J* = 7.0 Hz, 1H), 3.46 (s, 3H), 3.29 (s, 6H), 3.21 (s, 3H), 3.08 (s, 3H), 2.67 (s, 3H), 2.65 (s, 3H), 2.50–2.35 (m, 2H), 2.25–1.80 (m, 6H), 1.99 (s, 3H), 1.75–1.55 (m, 3H), 1.50–0.75 (m, 57H); ESI MS *m/z* 1233 [C<sub>62</sub>H<sub>109</sub>N<sub>11</sub>O<sub>14</sub> + H]<sup>+</sup>.

### **Example 3 – Preparation of Cyclosporin Alkyne**

**[0050]** To a stirred solution of acetyl cyclosporin aldehyde from Example 2 (1.94 g, 1.57 mmol) in methanol (20 mL) was added a solution of dimethyl (1-diazo-2-oxopropyl)phosphonate (3.01 g, 15.7 mmol) in methanol (10 mL) followed by potassium carbonate (2.17 g, 15.7 mmol). The resulting green suspension was stirred at room temperature overnight. The solution was filtered through diatomaceous earth and the filtrate was concentrated. The residue was dissolved in ethyl acetate (300 mL) and washed with water (2 × 100 mL). The combined aqueous layers were extracted with ethyl acetate (100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated to dryness. Purification by semi-preparative HPLC gave cyclosporin alkyne (848 mg, 45%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.86 (d, *J* = 9.7 Hz, 1H), 7.62 (d, *J* = 9.3 Hz, 1H), 7.56 (d, *J* = 6.9 Hz, 1H), 7.34 (d, *J* = 7.7 Hz, 1H), 5.73–5.68 (m, 1H), 5.57–5.45 (m, 2H), 5.22–4.45 (m, 12H), 4.03–3.98 (m, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.24 (s, 3H), 3.09 (s, 3H), 3.08 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.50–0.64 (m, 66H); ESI MS *m/z* 1187 [C<sub>61</sub>H<sub>107</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>.

### **Example 4 – Preparation of Cyclosporin Alkyne**

**[0051]** To a –78°C solution of (trimethylsilyl)diazomethane (4.6 mL, 2.0 M solution in Et<sub>2</sub>O, 9.2 mmol) in THF (5 mL) was added *n*-BuLi (3.4 mL, 2.5 M

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solution in hexanes, 8.4 mmol) dropwise. The resulting yellow suspension was stirred for 30 min, and then a solution of acetyl cyclosporine aldehyde from Example 2 (1.03 g, 0.84 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at -78°C for 30 min then warmed to 0°C for 15 min. The reaction was quenched with saturated NH<sub>4</sub>Cl. The mixture was partitioned between EtOAc and H<sub>2</sub>O. The aqueous layer was extracted with EtOAc. The combined organics were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave the cyclosporine alkyne (364 mg, 37%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.86 (d, *J* = 9.7 Hz, 1H), 7.62 (d, *J* = 9.3 Hz, 1H), 7.56 (d, *J* = 6.9 Hz, 1H), 7.34 (d, *J* = 7.7 Hz, 1H), 5.73–5.68 (m, 1H), 5.57–5.45 (m, 2H), 5.22–4.45 (m, 12H), 4.03–3.98 (m, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.24 (s, 3H), 3.09 (s, 3H), 3.08 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.50–0.64 (m, 66H); ESI MS *m/z* 1187 [C<sub>61</sub>H<sub>107</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>.

**Example 5 – Preparation of the Acetate of Cyclosporin Alkyne**

**[0052]** To a -78°C solution of (trimethylsilyl)diazomethane (4.5 mL, 2.0 M solution in Et<sub>2</sub>O, 8.9 mmol) in THF (10 mL) was added *n*-BuLi (3.2 mL, 2.5 M solution in hexanes, 8.1 mmol) dropwise. The resulting yellow suspension was stirred for 30 min, and then a solution of acetyl cyclosporine aldehyde from Example 2 (1.00 g, 0.81 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at -78°C for 5 min. The reaction was quenched with a mixture of acetic anhydride (1.5 mL, 4.1 mmol) and pyridine (1.4 mL, 4.9 mmol) in THF (5 mL) and then warmed to room temperature for 15 min. The reaction was quenched with saturated NH<sub>4</sub>Cl. The mixture was partitioned between Et<sub>2</sub>O and H<sub>2</sub>O. The aqueous layer was extracted with Et<sub>2</sub>O. The combined organics were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave the acetate of cyclosporine alkyne (389 mg, 40%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.46 (d, *J* = 9.5 Hz, 1H), 8.07 (d, *J* = 6.9 Hz, 1H), 7.72 (d, *J* = 9.1 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 5.69 (dd, *J* = 10.8, 3.6 Hz, 1H), 5.55–5.40 (m, 3H), 5.30 (dd, *J* = 11.7, 3.6 Hz, 1H), 5.15 (t, *J* = 6.1 Hz, 1H), 5.02–4.60 (m, 5H), 4.47 (t, *J* = 6.9 Hz, 1H), 3.46 (s, 3H), 3.28 (s, 3H), 3.23 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.69 (s, 3H),

2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.02 (m, 5H), 2.00 (s, 3H), 1.95–1.55 (m, 8H), 1.45–0.75 (m, 55H); ESI MS  $m/z$  1229 [ $C_{63}H_{109}N_{11}O_{13} + H$ ]<sup>+</sup>.

**Example 6 – Preparation of the Acetate of Cyclosporin Alkyne**

5 [0053] To a solution of cyclosporine alkyne from Example 3 (0.44 g, 0.37 mmol) in methylene chloride (5 mL) was added pyridine (0.90 mL, 11.1 mmol) followed by DMAP (68 mg, 0.55 mmol) and acetic anhydride (0.70 mL, 7.4 mmol), then the mixture was stirred at room temperature for 1.5 d. The reaction mixture was  
10 diluted with ethyl ether (100 mL), washed with a saturated solution of sodium bicarbonate (30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporine alkyne (0.23 g, 51%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.46 (d,  $J$  = 9.5 Hz, 1H), 8.07 (d,  $J$  =  
15 6.9 Hz, 1H), 7.72 (d,  $J$  = 9.1 Hz, 1H), 7.54 (d,  $J$  = 7.7 Hz, 1H), 5.69 (dd,  $J$  = 10.8, 3.6 Hz, 1H), 5.55–5.40 (m, 3H), 5.30 (dd,  $J$  = 11.7, 3.6 Hz, 1H), 5.15 (t,  $J$  = 6.1 Hz, 1H), 5.02–4.60 (m, 5H), 4.47 (t,  $J$  = 6.9 Hz, 1H), 3.46 (s, 3H), 3.28 (s, 3H), 3.23 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.02 (m, 5H), 2.00 (s, 3H), 1.95–1.55 (m, 8H), 1.45–0.75 (m, 55H); ESI MS  $m/z$  1229  
20 [ $C_{63}H_{109}N_{11}O_{13} + H$ ]<sup>+</sup>.

**Example 7 – Preparation of Cyclosporin yne-ene**

[0054] To a mixture of cyclosporin alkyne from Example 3 (55 mg, 0.046 mmol), copper(I) iodide (4 mg, 0.023 mmol),  
25 dichlorobis(triphenylphosphine)palladium(II) (16 mg, 0.023 mmol) in triethylamine (2 mL) was added vinyl iodide (34 μL, 0.46 mmol), then the mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative  
30 HPLC to afford cyclosporin yne-ene (24 mg, 43%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.11 (d,  $J$  = 9.3 Hz, 1H), 7.75 (d,  $J$  = 7.2 Hz, 1H), 7.56 (d,  $J$  = 8.4 Hz, 1H), 7.30 (d,  $J$  = 7.8 Hz, 1H), 5.80–5.65 (m, 3H), 5.56 (d,  $J$  = 2.4 Hz, 1H), 5.52–5.36 (m, 4H), 5.30 (dd,  $J$  = 11.4, 3.6 Hz, 1H), 5.20–4.95 (m, 6H), 4.84 (t,  $J$  = 7.2 Hz, 2H), 4.72–4.60 (m, 2H), 4.53 (t,  $J$  = 7.2 Hz, 1H), 3.88 (t,  $J$  = 6.3 Hz, 1H), 3.50



(s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.45–2.35 (m, 2H), 2.20–1.80 (m, 8H), 1.75–1.55 (m, 5H), 1.45–0.75 (m, 48H); ESI MS  $m/z$  1213 [ $C_{63}H_{109}N_{11}O_{12} + H$ ] $^{+}$ ; HPLC 98.6% (AUC),  $t_R$  = 19.32 min.

#### 5 **Example 8 – Preparation of *trans*-Cyclosporin yne-ene**

[0055] To a mixture of cyclosporin alkyne from Example 3 (65 mg, 0.055 mmol), copper(I) iodide (5 mg, 0.028 mmol), dichlorobis(triphenylphosphine)palladium(II) (20 mg, 0.028 mmol) in triethylamine  
10 (2 mL) was added *trans*-1,2-dichloroethylene (85  $\mu$ L, 1.1 mmol), then the mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford *trans*-cyclosporin yne-ene (13 mg, 19%) as a white solid:  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  8.08 (d,  $J$  = 9.6 Hz, 1H), 7.71 (d,  $J$  = 7.2 Hz, 1H), 7.57 (d,  $J$  = 8.1 Hz, 1H), 7.27 (d,  $J$  = 7.8 Hz, 1H), 6.43 (d,  $J$  = 13.6 Hz, 1H), 5.90  
15 (d,  $J$  = 13.6 Hz, 1H), 5.70 (dd,  $J$  = 10.8, 3.6 Hz, 1H), 5.45 (d,  $J$  = 6.3 Hz, 1H), 5.30 (dd,  $J$  = 11.7, 3.6 Hz, 1H), 5.17–4.92 (m, 5H), 4.83 (t,  $J$  = 6.9 Hz, 1H), 4.75–4.62 (m, 2H), 4.54 (t,  $J$  = 7.2 Hz, 1H), 3.85 (t,  $J$  = 6.3 Hz, 1H), 3.49 (s, 3H), 3.39 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.11 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.45–2.35 (m, 2H), 2.20–  
20 1.80 (m, 8H), 1.75–1.55 (m, 5H), 1.45–0.75 (m, 53H); ESI MS  $m/z$  1247 [ $C_{63}H_{108}ClN_{11}O_{12} + H$ ] $^{+}$ ; HPLC >99% (AUC),  $t_R$  = 19.92 min.

#### **Example 9 – Preparation of the Acetate of *cis*-Cyclosporin yne-ene**

25 [0056] To a mixture of the acetate of cyclosporine alkyne from Example 6 (166 mg, 0.14 mmol), copper(I) iodide (13 mg, 0.068 mmol), dichlorobis(triphenylphosphine)palladium(II) (48 mg, 0.068 mmol) in triethylamine (4 mL) was added *cis*-1,2-dichloroethylene (0.20 mL, 2.7 mmol), then the mixture was stirred at room temperature for 12 h. *Cis*-1,2-dichloroethylene (0.10 mL,  
30 1.3 mmol) was refilled, and the mixture was stirred for 5 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of *cis*-cyclosporin yne-ene (22 mg, 13%) as a white solid:  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  8.45 (d,  $J$  = 9.6 Hz, 1H), 8.08 (d,  $J$  = 7.2 Hz, 1H), 7.79 (d,  $J$  = 8.1 Hz, 1H), 7.59 (d,  $J$  = 7.8 Hz, 1H), 6.28

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(d,  $J = 7.4$  Hz, 1H), 5.82 (d,  $J = 7.4$  Hz, 1H), 5.70 (dd,  $J = 10.8, 3.6$  Hz, 1H), 5.62–5.10 (m, 5H), 5.03–4.72 (m, 4H), 4.64 (d,  $J = 13.8$  Hz, 1H), 4.48 (t,  $J = 7.0$  Hz, 1H), 3.44 (s, 3H), 3.29 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 7H), 1.99 (s, 3H), 1.95–1.60 (m, 5H), 1.45–0.75 (m, 55H); ESI MS  $m/z$  1289 [ $C_{65}H_{110}ClN_{11}O_{13} + H$ ] $^{+}$ .

### **Example 10 – Preparation of *cis*-Cyclosporin yne-ene**

[0057] To a solution of acetate of *cis*-cyclosporin yne-ene from Example 9 (22 mg, 0.017 mmol) in MeOH (3 mL) was added potassium carbonate (47 mg, 0.34 mmol), then the mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with ethyl acetate (30 mL), then washed with water (10 mL). The aqueous layer was separated and extracted with ethyl acetate (30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford *cis*-cyclosporin yne-ene (16 mg, 76%) as a white solid:  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  8.13 (d,  $J = 9.6$  Hz, 1H), 7.76 (d,  $J = 7.2$  Hz, 1H), 7.60 (d,  $J = 8.3$  Hz, 1H), 7.33 (d,  $J = 7.8$  Hz, 1H), 6.30 (d,  $J = 7.3$  Hz, 1H), 5.83 (d,  $J = 7.3$  Hz, 1H), 5.71 (dd,  $J = 11.1, 4.0$  Hz, 1H), 5.44 (d,  $J = 6.7$  Hz, 1H), 5.31 (dd,  $J = 11.5, 3.6$  Hz, 1H), 5.17–4.95 (m, 5H), 4.84 (t,  $J = 7.2$  Hz, 1H), 4.75–4.62 (m, 2H), 4.54 (t,  $J = 7.2$  Hz, 1H), 3.92 (t,  $J = 6.5$  Hz, 1H), 3.49 (s, 3H), 3.39 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.71 (s, 3H), 2.45–2.25 (m, 2H), 2.20–1.90 (m, 6H), 1.80–1.55 (m, 5H), 1.45–0.75 (m, 55H); ESI MS  $m/z$  1247 [ $C_{63}H_{108}ClN_{11}O_{12} + H$ ] $^{+}$ ; HPLC >99% (AUC),  $t_R = 19.21$  min.

### **Example 11 – Preparation of the Acetate of *trans*-Cyclosporin yne-ene**

[0058] To a mixture of the acetate of cyclosporin alkyne from Example 6 (74 mg, 0.06 mmol), copper(I) iodide (6 mg, 0.03 mmol), dichlorobis(triphenylphosphine)palladium(II) (21 mg, 0.03 mmol) in triethylamine (2 mL) was added (2-bromovinyl)trimethylsilane (0.18 mL, 1.2 mmol), then the mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of *trans*-cyclosporin yne-ene

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(16 mg, 20%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.48 (d,  $J = 9.6$  Hz, 1H), 8.07 (d,  $J = 7.2$  Hz, 1H), 7.66 (d,  $J = 8.1$  Hz, 1H), 7.54 (d,  $J = 7.8$  Hz, 1H), 6.24 (d,  $J = 19.2$  Hz, 1H), 5.88 (d,  $J = 19.2$  Hz, 1H), 5.70 (dd,  $J = 10.8, 3.6$  Hz, 1H), 5.55–5.10 (m, 6H), 5.03–4.92 (m, 2H), 4.86 (t,  $J = 7.2$  Hz, 1H), 4.76 (t,  $J = 9.5$  Hz, 1H), 4.64 (d,  $J = 13.9$  Hz, 1H), 4.46 (t,  $J = 7.2$  Hz, 1H), 3.44 (s, 3H), 3.31 (s, 3H), 3.25 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 7H), 1.98 (s, 3H), 1.75–1.55 (m, 3H), 1.45–0.75 (m, 56H), 0.07 (s, 9H); ESI MS  $m/z$  1327 [ $\text{C}_{68}\text{H}_{119}\text{N}_{11}\text{O}_{13}\text{Si} + \text{H}$ ] $^+$ .

#### 10 **Example 12 – Preparation of *trans*-Cyclosporin yne-ene**

[0059] To a solution of the acetate of *trans*-cyclosporin yne-ene from Example 11 (16 mg, 0.012 mmol) in MeOH (2 mL) was added potassium carbonate (41 mg, 0.30 mmol), then the mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with ethyl acetate (30 mL), then washed with water (10 mL). The aqueous layer was separated and extracted with ethyl acetate (30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford *trans*-cyclosporin yne-ene (6 mg, 40%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 (d,  $J = 9.8$  Hz, 1H), 7.78 (d,  $J = 7.2$  Hz, 1H), 7.58 (d,  $J = 8.2$  Hz, 1H), 7.34 (d,  $J = 7.9$  Hz, 1H), 6.30 (d,  $J = 19.2$  Hz, 1H), 5.89 (d,  $J = 19.2$  Hz, 1H), 5.71 (dd,  $J = 11.3, 4.1$  Hz, 1H), 5.39 (d,  $J = 7.1$  Hz, 1H), 5.30 (dd,  $J = 11.4, 3.4$  Hz, 1H), 5.20–4.95 (m, 5H), 4.83 (t,  $J = 7.2$  Hz, 1H), 4.75–4.65 (m, 2H), 4.53 (t,  $J = 7.2$  Hz, 1H), 3.92 (t,  $J = 6.4$  Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.71 (s, 3H), 2.60–2.35 (m, 2H), 2.20–1.55 (m, 12H), 1.45–0.75 (m, 54H), 0.07 (s, 9H); ESI MS  $m/z$  1285 [ $\text{C}_{66}\text{H}_{117}\text{N}_{11}\text{O}_{12}\text{Si} + \text{H}$ ] $^+$ ; HPLC 98.5% (AUC),  $t_R = 22.71$  min.

#### 30 **Example 13 – Preparation of *trans*-Cyclosporin yne-ene**

[0060] To a mixture of cyclosporine alkyne from Example 3 (40 mg, 0.03 mmol) in triethylamine (2 mL) and tetrahydrofuran (1 mL) was added dichlorobis(triphenylphosphine)palladium(II) (15 mg, 0.02 mmol), copper(I) iodide (4 mg, 0.02 mmol) and *trans*-1-bromopropene (50  $\mu\text{L}$ , 0.6 mmol), then the mixture

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was stirred at room temperature for 3 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification twice by semi-preparative HPLC gave *trans*-cyclosporin yne-ene (5.5 mg, 15%) as a yellow solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.10 (d, *J* = 5.6 Hz, 1H), 7.75–7.67 (m, 3H), 7.49–7.38 (m, 4H), 6.05–5.99 (m, 1H), 5.70 (dd, *J* = 10.9, 4.2 Hz, 1H), 5.42 (dd, *J* = 15.8, 1.8 Hz, 1H), 5.36 (d, *J* = 7.0 Hz, 1H), 5.28 (dd, *J* = 11.4, 3.5 Hz, 1H), 5.19–5.17 (m, 1H), 5.09 (t, *J* = 6.7 Hz, 1H), 5.06–4.98 (m, 3H), 4.84 (t, *J* = 7.2 Hz, 1H), 4.73–4.68 (m, 3H), 4.49 (t, *J* = 7.3 Hz, 1H), 3.90 (t, *J* = 6.6 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.55–1.80 (m, 12H), 1.75–1.56 (m, 13H), 1.50–1.19 (m, 41H); ESI MS *m/z* 1227 [C<sub>64</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>; HPLC >99% (AUC), *t<sub>R</sub>* = 19.76 min.

**Example 14 – Preparation of *cis*-Cyclosporin yne-ene**

**[0061]** A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), *cis*-1-bromopropene (300 μL, 3.5 mmol) and copper(I) iodide (14 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (51 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a brown solid. The solid was further purified twice by semi-preparative HPLC to afford *cis*-cyclosporin yne-ene (29 mg, 35%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.15 (d, *J* = 9.5 Hz, 1H), 7.77 (d, *J* = 7.3 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.27 (d, *J* = 6.4 Hz, 1H), 5.94–5.83 (m, 1H), 5.70 (dd, *J* = 11.0, 4.2 Hz, 1H), 5.45–5.39 (m, 2H), 5.29 (dd, *J* = 11.5, 3.8 Hz, 1H), 5.20–5.16 (m, 1H), 5.10–4.97 (m, 4H), 4.88–4.79 (m, 1H), 4.74–4.67 (m, 3H), 4.54–4.47 (m, 1H), 3.90 (t, *J* = 6.5 Hz, 1H), 3.50 (s, 3H), 3.39 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.48–0.80 (m, 70H); ESI MS *m/z* 1227 [C<sub>64</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>, HPLC >99% (AUC), *t<sub>R</sub>* = 19.85 min.

**Example 15 – Preparation of Cyclosporin yne-ene**

[0062] To a mixture of cyclosporin alkyne from Example 3 (40 mg, 0.03 mmol) in triethylamine (2 mL) and tetrahydrofuran (1 mL) was added  
5 dichlorobis(triphenylphosphine)palladium(II) (15 mg, 0.02 mmol), copper(I) iodide (4 mg, 0.02 mmol) and 2-bromopropene (50  $\mu$ L, 0.6 mmol), then the mixture was stirred at room temperature for 5 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification twice by semi-preparative HPLC gave cyclosporin yne-ene (4.5 mg, 12%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 (d,  $J = 9.0$  Hz, 1H), 7.74 (d,  $J = 7.3$  Hz, 1H), 7.49 (d,  $J = 8.3$  Hz, 1H), 7.22 (d,  $J = 7.8$  Hz, 1H), 5.71 (dd,  $J = 10.9, 4.3$  Hz, 1H), 5.41 (d,  $J = 6.8$  Hz, 1H), 5.35 (dt,  $J = 9.1, 5.8$  Hz, 1H), 5.27 (dd,  $J = 11.5, 3.7$  Hz, 1H), 5.20–5.18 (m, 3H), 5.14 (s, 1H), 5.08 (t,  $J = 6.9$  Hz, 1H), 5.05–4.98 (m, 2H), 4.84 (app quintet,  $J = 6.9$  Hz, 1H), 4.73–4.67 (m, 2H), 4.52 (app quintet,  $J = 7.4$  Hz, 1H), 3.88 (t,  $J = 6.5$  Hz, 1H),  
15 3.50 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.21–3.18 (m, 1H), 3.13 (s, 3H), 3.10 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.60 (dd,  $J = 17.1, 3.9$  Hz, 1H), 2.46–2.35 (m, 1H), 2.63 (t,  $J = 7.7$  Hz, 1H), 2.18–2.07 (m, 6H), 2.05–1.96 (m, 3H), 1.90–1.82 (m, 5H), 1.81–1.59 (m, 5H), 1.52–1.38 (m, 4H), 1.36–1.23 (m, 13H), 1.01–0.84 (m, 30H); ESI MS  $m/z$  1227 [ $\text{C}_{64}\text{H}_{111}\text{N}_{11}\text{O}_{12} + \text{H}$ ] $^+$ ; HPLC 98.8% (AUC),  $t_R = 19.82$  min.

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**Example 16 – Preparation of Cyclosporin yne-ene**

[0063] To a mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol) and copper(I) iodide (13 mg, 0.07 mmol) in triethylamine (3 mL) was  
25 added bromostyrene (a mixture of *cis* and *trans* isomers, 180  $\mu$ L, 1.4 mmol) then the mixture was stirred until a clear solution formed.  
Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was  
30 purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a brown solid. The solid was further purified twice by semi-preparative HPLC to afford cyclosporin yne-ene (17 mg, 19%) as a white solid and a mixture of isomers (*cis/trans*  $\sim 1:4$  by  $^1\text{H}$  NMR):  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.10 (d,  $J = 9.7$  Hz, 1H), 7.81 (d,  $J = 7.4$  Hz, 1H), 7.71 (d,  $J = 7.4$  Hz, 1H), 7.47 (d,  $J =$

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8.5 Hz, 1H), 7.38–7.29 (m, 12H), 6.94–6.83 (m, 4H), 5.70 (dd,  $J = 11.0, 4.3$  Hz, 1H), 5.44 (d,  $J = 6.9$  Hz, 2H), 5.19–5.15 (m, 2H), 5.08 (t,  $J = 7.3$  Hz, 1H), 5.06–5.00 (m, 6H), 4.83 (t,  $J = 7.7$  Hz, 1H), 4.73–4.69 (m, 4H), 4.55–4.40 (m, 3H), 3.92 (t,  $J = 6.6$  Hz, 1H), 3.63 (s, 3H), 3.52 (s, 3H), 3.40 (s, 3H), 3.29 (s, 3H), 3.12 (s, 3H), 3.11 (s, 3H), 2.50–1.87 (m, 10H), 1.84–1.57 (m, 3H), 1.55–1.19 (m, 10H), 1.08–0.70 (m, 31H); ESI MS  $m/z$  1289 [ $C_{69}H_{113}N_{11}O_{12} + H$ ]<sup>+</sup>; HPLC 98.2% (AUC),  $t_R = 20.66$  min.

### **Example 17 – Preparation of Phenyl Cyclosporin Alkyne**

10   **[0064]**       A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol) in triethylamine (3 mL) was degassed with N<sub>2</sub> for 5 min. Dichlorobis(triphenylphosphine)palladium(II) (28 mg, 0.04 mmol), copper(I) iodide (8 mg, 0.04 mmol) and iodobenzene (80  $\mu$ L, 0.70 mmol) were added, then the mixture was stirred at room temperature for 1.5 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification by semi-preparative HPLC gave cyclosporine phenyl alkyne (7 mg, 8%) as a brown solid: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.18 (d,  $J = 9.8$  Hz, 1H), 7.78 (d,  $J = 7.4$  Hz, 1H), 7.71–7.68 (m, 1H), 7.51 (d,  $J = 8.3$  Hz, 1H), 7.40–7.37 (m, 4H), 7.20 (d,  $J = 7.9$  Hz, 1H), 5.69 (dd,  $J = 10.6, 3.7$  Hz, 1H), 5.45 (d,  $J = 6.9$  Hz, 1H), 5.32–5.27 (m, 1H), 5.20–5.16 (m, 1H), 5.09–4.97 (m, 4H), 4.83 (t,  $J = 7.1$  Hz, 1H), 4.74–4.67 (m, 2H), 4.52 (t,  $J = 7.3$  Hz, 1H), 3.92 (t,  $J = 6.5$  Hz, 1H), 3.53 (s, 3H), 3.40 (s, 3H), 3.30 (s, 3H), 3.22–3.17 (m, 2H), 3.13 (s, 3H), 3.10 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.53–2.35 (m, 2H), 2.29–1.91 (m, 10H), 1.83–1.83 (m, 11H), 1.50–1.18 (m, 11H), 1.10–0.76 (m, 32H); ESI MS  $m/z$  1263 [ $C_{67}H_{111}N_{11}O_{12} + H$ ]<sup>+</sup>; HPLC >99% (AUC),  $t_R = 20.01$  min.

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### **Example 18 – Preparation of 4-Methoxyphenyl Cyclosporin Alkyne**

**[0065]**       A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), 1-iodo-4-methoxybenzene (150  $\mu$ L, 1.4 mmol) and copper(I) iodide (13 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl

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acetate) to give a brown solid. The solid was further purified twice by semi-preparative HPLC to afford 4-methoxyphenyl cyclosporin alkyne (23 mg, 26%) as a white solid:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  8.17 (d,  $J = 9.7$  Hz, 1H), 7.77 (d,  $J = 7.4$  Hz, 1H), 7.50 (d,  $J = 8.5$  Hz, 1H), 7.31 (d,  $J = 8.8$  Hz, 2H), 7.21 (d,  $J = 7.9$  Hz, 1H), 6.80 (d,  $J = 8.8$  Hz, 2H), 5.68 (dd,  $J = 11.0, 4.3$  Hz, 1H), 5.42 (d,  $J = 7.1$  Hz, 1H), 5.29 (dd,  $J = 11.6, 3.9$  Hz, 1H), 5.20–5.18 (m, 2H), 5.09–5.05 (m, 2H), 5.01 (dd,  $J = 16.2, 8.1$  Hz, 1H), 4.85 (app quintet,  $J = 6.9$  Hz, 1H), 4.73–4.69 (m, 2H), 4.51 (app quintet,  $J = 7.2$  Hz, 1H), 3.92 (d,  $J = 6.4$  Hz, 1H), 3.80 (s, 3H), 3.52 (s, 3H), 3.40 (s, 3H), 3.29 (s, 3H), 3.20–3.14 (m, 6H), 3.13 (s, 3H), 3.10 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.44–1.95 (m, 12H), 1.76–1.61 (m, 3H), 1.45–1.24 (m, 16H), 1.07 (t,  $J = 8.0$  Hz, 4H), 0.97–0.79 (m, 27H); ESI MS  $m/z$  1293 [ $\text{C}_{68}\text{H}_{113}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ ; HPLC >99% (AUC),  $t_R = 19.59$  min.

#### **Example 19 – Preparation of 4-Fluorophenyl Cyclosporin Alkyne**

**[0066]** A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), 4-fluoro-1-iodobenzene (160  $\mu\text{L}$ , 1.4 mmol) and copper(I) iodide (14 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a brown solid. The solid was further purified twice by semi-preparative HPLC to afford 4-fluorophenyl cyclosporin alkyne (8.9 mg, 10%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.14 (d,  $J = 9.8$  Hz, 1H), 7.76 (d,  $J = 7.4$  Hz, 1H), 7.52 (d,  $J = 8.4$  Hz, 1H), 7.35 (ddd,  $J = 8.7, 5.4, 2.0$  Hz, 2H), 7.19 (d,  $J = 7.9$  Hz, 1H), 6.97 (dd,  $J = 8.7, 8.7$  Hz, 2H), 5.68 (dd,  $J = 11.1, 4.1$  Hz, 1H), 5.45 (d,  $J = 6.7$  Hz, 1H), 5.27 (dd,  $J = 11.5, 3.7$  Hz, 1H), 5.18–5.14 (m, 1H), 5.09–4.94 (m, 4H), 4.88–4.79 (m, 1H), 4.74–4.66 (m, 2H), 4.56–4.47 (m, 1H), 3.91 (t,  $J = 6.4$  Hz, 1H), 3.52 (s, 3H), 3.40 (s, 3H), 3.29 (s, 3H), 3.13 (s, 3H), 3.10 (s, 3H), 2.71 (s, 3H), 2.69 (s, 3H), 2.45–0.76 (m, 68H); ESI MS  $m/z$  1280 [ $\text{C}_{67}\text{H}_{110}\text{FN}_{11}\text{O}_{12} + \text{H}$ ] $^+$ ; HPLC >99% (AUC),  $t_R = 20.18$  min.

**Example 20 – Preparation of Thiophen-2-yl Cyclosporin Alkyne**

[0067] A mixture of cyclosporin alkyne from Example 3 (80 mg, 0.07 mmol), 2-iodothiophene (328 mg, 1.4 mmol) and copper(I) iodide (13 mg, 0.07 mmol) in triethylamine (3 mL) was stirred until a clear solution formed. Dichlorobis(triphenylphosphine)palladium(II) (50 mg, 0.07 mmol) was added, and then the mixture was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The residue was purified by column chromatography (silica gel, 7:3 hexanes/ethyl acetate to ethyl acetate) to give a light brown solid. The solid was further purified twice by semi-preparative HPLC to afford thiophen-2-yl cyclosporin alkyne (10.7 mg, 12%) as a white solid: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.07 (d, *J* = 9.6 Hz, 1H), 7.74 (d, *J* = 7.4 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.25 (d, *J* = 5.6 Hz, 1H), 7.15 (dd, *J* = 5.2, 1.0 Hz, 1H), 7.11 (dd, *J* = 3.5, 0.9 Hz, 1H), 6.92 (dd, *J* = 5.1, 3.6 Hz, 1H), 5.69 (dd, *J* = 11.0, 4.3 Hz, 1H), 5.47 (d, *J* = 6.4 Hz, 1H), 5.29 (dd, *J* = 11.4, 3.6 Hz, 1H), 5.18–5.16 (m, 1H), 5.07 (t, *J* = 6.9 Hz, 1H), 5.05–5.00 (m, 2H), 4.86–4.81 (m, 1H), 4.74–4.67 (m, 2H), 4.57–4.51 (m, 1H), 3.90 (t, *J* = 6.4 Hz, 1H), 3.52 (s, 3H), 3.39 (s, 3H), 3.28 (s, 3H), 3.25–3.15 (m, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 3.07–2.90 (m, 4H), 2.82–2.75 (m, 1H), 2.72 (s, 3H), 2.70 (s, 3H), 2.44–1.90 (m, 8H), 1.79–1.58 (m, 6H), 1.48–1.18 (m, 11H), 1.05–0.80 (m, 36H); ESI MS *m/z* 1269 [C<sub>65</sub>H<sub>109</sub>N<sub>11</sub>O<sub>12</sub>S + H]<sup>+</sup>; HPLC 98.8% (AUC), *t<sub>R</sub>* = 19.76 min.

**Example 21 – Preparation of the Acetate of Cyclosporin Diyne**

[0068] To a solution of the acetate of cyclosporin alkyne from Example 6 (90 mg, 0.073 mmol) in pyrrolidine (1 mL) were added copper(I) iodide (7 mg, 0.037 mmol) and dichlorobis(triphenylphosphine)palladium(II) (26 mg, 0.037 mmol), then the mixture was stirred for 5 min at room temperature. 1-Butynyl iodide (145 μL, 1.46 mmol) was added dropwise, and then the mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (40 mL) and washed with a saturated solution of ammonium chloride (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of



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cyclosporin diyne (15 mg, 16%) as a brown solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.48 (d,  $J = 9.6$  Hz, 1H), 8.05 (d,  $J = 6.9$  Hz, 1H), 7.60 (d,  $J = 9.0$  Hz, 1H), 7.55 (d,  $J = 7.8$  Hz, 1H), 5.70 (dd,  $J = 10.8, 3.9$  Hz, 1H), 5.58–5.35 (m, 2H), 5.30 (dd,  $J = 12.0, 3.3$  Hz, 2H), 5.15 (t,  $J = 6.9$  Hz, 1H), 5.05–4.80 (m, 3H), 4.73 (t,  $J = 9.6$  Hz, 1H), 4.64 (d,  $J = 13.8$  Hz, 1H), 4.44 (t,  $J = 6.9$  Hz, 1H), 3.43 (s, 3H), 3.32 (s, 3H), 3.27 (s, 3H), 3.20 (s, 3H), 3.07 (s, 3H), 2.68 (s, 3H), 2.66 (s, 3H), 2.50–2.35 (m, 1H), 2.30–1.80 (m, 10H), 2.04 (s, 3H), 1.75–1.55 (m, 3H), 1.45–0.75 (m, 59H); ESI MS  $m/z$  1281  $[\text{C}_{67}\text{H}_{113}\text{N}_{11}\text{O}_{13} + \text{H}]^+$ .

#### 10 **Example 22 – Preparation of Cyclosporin Diyne**

[0069] To a solution of the acetate of cyclosporin diyne from Example 21 (18 mg, 0.014 mmol) in MeOH (2 mL) was added potassium carbonate (39 mg, 0.28 mmol), then the mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate ( $3 \times 30$  mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diyne (9 mg, 53%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.98 (d,  $J = 9.3$  Hz, 1H), 7.73 (d,  $J = 7.2$  Hz, 1H), 7.39 (d,  $J = 8.4$  Hz, 1H), 7.23 (d,  $J = 8.1$  Hz, 1H), 5.71 (dd,  $J = 10.8, 3.9$  Hz, 1H), 5.41 (d,  $J = 6.6$  Hz, 1H), 5.28 (dd,  $J = 11.7, 3.9$  Hz, 1H), 5.20–4.95 (m, 5H), 4.83 (t,  $J = 7.2$  Hz, 1H), 4.78–4.63 (m, 2H), 4.52 (t,  $J = 7.2$  Hz, 1H), 3.90 (t,  $J = 6.3$  Hz, 1H), 3.50 (s, 3H), 3.37 (s, 3H), 3.28 (s, 3H), 3.12 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.55–2.05 (m, 7H), 1.90–0.80 (m, 66H); ESI MS  $m/z$  1238  $[\text{C}_{65}\text{H}_{111}\text{N}_{11}\text{O}_{12} + \text{H}]^+$ ; HPLC >99% (AUC),  $t_R = 20.06$  min.

#### **Example 23 – Preparation of the Acetate of Cyclosporin Alkynyl Bromide**

[0070] To a solution of the acetate of cyclosporin alkyne from Example 6 (0.22 g, 0.18 mmol) in acetone (5 mL) was added *N*-bromosuccinimide (64 mg, 0.36 mmol) followed by silver nitrate (6 mg, 0.036 mmol). The reaction flask was wrapped with aluminum foil. The reaction mixture was stirred at room temperature for 1 h, then poured into ice-water (20 mL) and extracted with ethyl ether ( $3 \times 40$  mL). The combined organics were washed with brine (30 mL), dried over

anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporin alkynyl bromide (0.23 g, 98%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.48 (d,  $J$  = 9.6 Hz, 1H), 8.07 (d,  $J$  = 6.9 Hz, 1H), 7.80 (d,  $J$  = 8.9 Hz, 1H), 7.63 (d,  $J$  = 7.7 Hz, 1H), 5.70 (dd,  $J$  = 10.8, 3.6 Hz, 1H), 5.60–5.15 (m, 5H), 5.02–4.80 (m, 4H), 4.76 (d,  $J$  = 9.3 Hz, 1H), 4.65 (d,  $J$  = 13.9 Hz, 1H), 4.48 (t,  $J$  = 7.0 Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.25 (s, 3H), 3.19 (s, 3H), 3.10 (s, 3H), 2.69 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 7H), 2.02 (s, 3H), 1.75–1.55 (m, 4H), 1.45–0.75 (m, 55H); ESI MS  $m/z$  1307 [ $\text{C}_{63}\text{H}_{108}\text{BrN}_{11}\text{O}_{13} + \text{H}$ ] $^+$ .

10

**Example 24 – Preparation of the Acetate of Cyclosporin(trimethylsilyl)diyne**

[0071] To a solution of the acetate of cyclosporin alkynyl bromide from Example 23 (20 mg, 0.015 mmol) in pyrrolidine (1 mL) was added

15 (trimethylsilyl)acetylene (42  $\mu\text{L}$ , 0.30 mmol) followed by copper(I) iodide (3 mg, 0.015 mmol) and dichlorobis(triphenylphosphine)palladium(II) (6 mg, 0.008 mmol), then the mixture was stirred at room temperature for 1 h. The reaction was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3  $\times$  30 mL). The combined organics were dried over anhydrous sodium sulfate and  
20 concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporin (trimethylsilyl)diyne (6 mg, 30%) as a pale-brown solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.48 (d,  $J$  = 9.6 Hz, 1H), 8.05 (d,  $J$  = 6.7 Hz, 1H), 7.65 (d,  $J$  = 8.8 Hz, 1H), 7.57 (d,  $J$  = 7.9 Hz, 1H), 5.70 (dd,  $J$  = 11.2, 3.8 Hz, 1H), 5.55–5.35 (m, 2H), 5.29 (td,  $J$  = 11.9, 3.7 Hz, 2H), 5.16 (d,  $J$  = 6.1 Hz, 1H), 5.03–4.80 (m, 3H), 4.72 (t,  $J$  = 9.4 Hz, 1H), 4.63 (d,  $J$  = 13.9 Hz, 1H), 4.46 (t,  $J$  = 7.0 Hz, 1H), 3.42 (s, 3H), 3.32 (s, 3H), 3.27 (s, 3H), 3.20 (s, 3H), 3.08 (s, 3H), 2.68 (s, 3H), 2.67 (s, 3H), 2.45–2.05 (m, 7H), 2.04 (s, 3H), 1.75–1.55 (m, 3H), 1.45–0.75 (m, 56H), 0.15 (s, 9H); ESI MS  $m/z$  1325 [ $\text{C}_{68}\text{H}_{117}\text{N}_{11}\text{O}_{13}\text{Si} + \text{H}$ ] $^+$ .

30 **Example 25 – Preparation of Cyclosporin Diyne**

[0072] To a solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (9 mg, 0.007 mmol) in MeOH (2 mL) was added potassium carbonate (19 mg, 0.14 mmol), then the mixture was stirred overnight at room temperature. The

reaction mixture was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporine diyne (6 mg, 71%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.95 (d, *J* = 9.7 Hz, 1H), 7.65 (d, *J* = 7.4 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 1H), 5.71 (dd, *J* = 10.8, 3.8 Hz, 1H), 5.48 (d, *J* = 6.2 Hz, 1H), 5.27 (dd, *J* = 11.5, 3.9 Hz, 1H), 5.15–4.95 (m, 5H), 4.83 (t, *J* = 7.1 Hz, 1H), 4.75–4.62 (m, 2H), 4.53 (t, *J* = 7.2 Hz, 1H), 3.89 (t, *J* = 6.2 Hz, 1H), 3.51 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.11 (s, 3H), 3.10 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.60–2.35 (m, 2H), 2.20–0.80 (m, 67H); ESI MS *m/z* 1211 [C<sub>63</sub>H<sub>107</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>; HPLC 98.5% (AUC), *t<sub>R</sub>* = 18.78 min.

#### **Example 26 – Preparation of the Acetate of Cyclosporin Diyne**

**[0073]** To an ice-cooled solution of 1-propynylmagnesium bromide (0.54 mL, 0.5 M in THF, 0.27 mmol) in THF (1 mL) was added a solution of zinc chloride (0.27 mL, 1 M in ethyl ether, 0.27 mmol). The reaction was stirred at 0°C for 10 min, and then allowed to warm to room temperature. A solution of the acetate of cyclosporin alkynyl bromide from Example 23 (35 mg, 0.027 mmol) in THF (1 mL) was added into the reaction mixture followed by dichlorobis(triphenylphosphine)palladium(II) (10 mg, 0.014 mmol). The resulting reaction mixture was stirred at room temperature for 1.5 h, and then quenched with a saturated solution of ammonium chloride (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the acetate of cyclosporin diyne (14 mg, 41%) as a pale-brown solid: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.42 (d, *J* = 9.5 Hz, 1H), 8.07 (d, *J* = 7.0 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H), 5.71 (dd, *J* = 11.0, 4.5 Hz, 1H), 5.58–5.37 (m, 3H), 5.27 (dd, *J* = 12.0, 4.0 Hz, 1H), 5.17 (t, *J* = 6.0 Hz, 1H), 5.03–4.93 (m, 3H), 4.87 (t, *J* = 7.0 Hz, 1H), 4.81 (t, *J* = 9.5 Hz, 1H), 4.65 (d, *J* = 13.5 Hz, 1H), 4.49 (t, *J* = 7.0 Hz, 1H), 3.43 (s, 3H), 3.29 (s, 3H), 3.23 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.28–2.04 (m, 7H),

2.03 (s, 3H), 2.02–1.92 (m, 2H), 1.88 (s, 3H), 1.75–1.62 (m, 4H), 1.45–0.75 (m, 53H); ESI MS  $m/z$  1267 [ $C_{66}H_{111}N_{11}O_{13} + H$ ]<sup>+</sup>.

**Example 27 – Preparation of Cyclosporin Diyne**

5 [0074] To a solution of the acetate of cyclosporin diyne from Example 26 (14 mg, 0.011 mmol) in MeOH (2 mL) was added potassium carbonate (30 mg, 0.22 mmol), then the mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated solution of ammonium chloride, and then  
10 extracted with ethyl acetate (3 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diyne (8 mg, 62%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.93 (d,  $J$  = 9.5 Hz, 1H), 7.71 (d,  $J$  = 7.0 Hz, 1H), 7.51 (d,  $J$  = 8.2 Hz, 1H), 7.37 (d,  $J$  = 7.4 Hz, 1H), 5.71 (dd,  $J$  = 11.0, 2.9 Hz, 1H), 5.41 (d,  $J$  = 6.5 Hz, 1H), 5.38–5.27 (m, 1H), 5.15–4.95 (m, 4H), 4.84 (t,  $J$  = 7.0 Hz, 1H), 4.75–4.65 (m, 2H), 4.54 (t,  $J$  = 6.9 Hz, 1H), 3.94 (t,  $J$  = 6.5 Hz, 1H), 3.48 (s, 3H), 3.37 (s, 3H), 3.25 (s, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.71 (s, 3H), 2.55–2.05 (m, 7H), 1.88 (s, 3H), 1.75–0.75 (m, 62H); ESI MS  $m/z$  1225 [ $C_{64}H_{109}N_{11}O_{12} + H$ ]<sup>+</sup>; HPLC >99% (AUC),  $t_R$  = 19.38 min.

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**Example 28 – Preparation of the Acetate of Cyclosporin Cyclopropyl Diyne**

[0075] To an ice-cooled solution of cyclopropyl(trimethylsilyl)acetylene (37 mg, 0.27 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride  
25 (0.32 mL, 1 M in THF, 0.32 mmol), then the mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then a solution of the acetate of cyclosporin alkynyl bromide from Example 23 (35 mg, 0.027 mmol) in triethylamine (1 mL) was added into the mixture followed by copper(I) iodide (3 mg, 0.014 mmol) and dichlorobis(triphenylphosphine)palladium(II) (10 mg, 0.014 mmol).  
30 The resulting reaction mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with a saturated solution of ammonium chloride (10 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative

HPLC to afford the acetate of cyclosporin cyclopropyl diyne (23 mg, 66%) as a pale-brown solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.45 (d,  $J = 9.4$  Hz, 1H), 8.05 (d,  $J = 6.8$  Hz, 1H), 7.71 (d,  $J = 9.0$  Hz, 1H), 7.60 (d,  $J = 7.8$  Hz, 1H), 5.70 (dd,  $J = 10.9$ , 3.6 Hz, 1H), 5.58–5.23 (m, 4H), 5.16 (d,  $J = 5.5$  Hz, 1H), 5.02–4.80 (m, 3H), 4.77 (t,  $J = 9.6$  Hz, 1H), 4.64 (d,  $J = 13.9$  Hz, 1H), 4.46 (t,  $J = 7.0$  Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.25 (s, 3H), 3.19 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.03 (m, 6H), 2.02 (s, 3H), 1.75–1.60 (m, 3H), 1.45–0.70 (m, 63H); ESI MS  $m/z$  1293 [ $\text{C}_{68}\text{H}_{113}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ .

### 10 **Example 29 – Preparation of Cyclosporin Cyclopropyl Diyne**

[0076] To a solution of the acetate of cyclosporin cyclopropyl diyne from Example 28 (20 mg, 0.015 mmol) in MeOH (2 mL) was added potassium carbonate (41 mg, 0.30 mmol), then the mixture was stirred overnight at room temperature. The reaction mixture was quenched with a saturated solution of ammonium chloride, and then extracted with ethyl acetate (3  $\times$  30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin cyclopropyl diyne (16 mg, 84%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.97 (d,  $J = 9.7$  Hz, 1H), 7.70 (d,  $J = 7.3$  Hz, 1H), 7.40 (d,  $J = 8.4$  Hz, 1H), 7.25 (d,  $J = 8.7$  Hz, 1H), 5.71 (dd,  $J = 11.0$ , 4.0 Hz, 1H), 5.41 (d,  $J = 6.6$  Hz, 1H), 5.28 (dd,  $J = 11.4$ , 3.9 Hz, 1H), 5.15–4.95 (m, 4H), 4.83 (t,  $J = 7.2$  Hz, 1H), 4.75–4.65 (m, 3H), 4.52 (t,  $J = 7.2$  Hz, 1H), 3.90 (t,  $J = 6.4$  Hz, 1H), 3.49 (s, 3H), 3.37 (s, 3H), 3.28 (s, 3H), 3.12 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.55–2.30 (m, 2H), 2.20–0.75 (m, 71H); ESI MS  $m/z$  1251 [ $\text{C}_{66}\text{H}_{111}\text{N}_{11}\text{O}_{12} + \text{H}$ ] $^+$ ; HPLC >99% (AUC),  $t_R = 19.98$  min.

### **Example 30 – Preparation of the Acetate of Cyclosporin Diyne**

[0077] A mixture of the acetate of cyclosporine alkynyl bromide from Example 23 (40 mg, 0.03 mmol), dichlorobis(triphenylphosphine)palladium(II) (14 mg, 0.02 mmol), copper(I) iodide (4 mg, 0.02 mmol) and phenylacetylene (30  $\mu\text{L}$ , 0.30 mmol) in triethylamine (2 mL) was stirred at room temperature overnight. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. Purification by column chromatography (silica gel, 100% hexanes followed by 100%

EtOAc) followed by semi-preparative HPLC gave the acetate of cyclosporin diyne (11 mg, 27%) as a brown solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.52 (d,  $J = 9.7$  Hz, 1H), 8.05 (d,  $J = 6.9$  Hz, 1H), 7.62–7.54 (m, 2H), 7.47–7.44 (m, 2H), 7.34–7.28 (m, 3H), 5.69 (dd,  $J = 10.9, 3.9$  Hz, 1H), 5.59–5.50 (m, 1H), 5.47–5.43 (m, 1H), 5.35 (t,  $J = 3.5$  Hz, 1H), 5.31 (t,  $J = 3.4$  Hz, 1H), 5.15 (t,  $J = 6.2$  Hz, 1H), 5.02–4.91 (m, 4H), 4.86 (t,  $J = 7.2$  Hz, 1H), 4.75 (t,  $J = 9.6$  Hz, 1H), 4.67–4.63 (m, 1H), 4.45 (t,  $J = 7.0$  Hz, 1H), 3.44 (s, 3H), 3.42 (s, 3H), 3.28 (s, 3H), 3.20 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.66 (s, 3H), 2.52–2.11 (m, 8H), 2.07 (s, 3H), 1.75–1.55 (m, 4H), 1.41–1.18 (m, 20H), 1.05–0.82 (m, 34H); ESI MS  $m/z$  1329 [ $\text{C}_{71}\text{H}_{113}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ .

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### **Example 31 – Preparation of Cyclosporin Diyne**

[0078] To a solution of the acetate of cyclosporin diyne from Example 30 (11 mg, 0.008 mmol) in MeOH (1 mL) was added potassium carbonate (11 mg, 0.08 mmol) and then the mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with  $\text{H}_2\text{O}$  (2  $\times$ ), brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. Purification by semi-preparative HPLC gave the cyclosporin diyne (6.6 mg, 64%) as a brown solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.98 (d,  $J = 9.7$  Hz, 1H), 7.69 (d,  $J = 7.5$  Hz, 1H), 7.50–7.47 (m, 2H), 7.41 (d,  $J = 8.3$  Hz, 1H), 7.32–7.28 (m, 3H), 7.22 (d,  $J = 7.9$  Hz, 1H), 5.70 (dd,  $J = 11.0, 4.1$  Hz, 1H), 5.48 (d,  $J = 6.4$  Hz, 1H), 5.34–5.29 (m, 2H), 5.15–4.99 (m, 6H), 4.83 (t,  $J = 7.0$  Hz, 1H), 4.74–4.66 (m, 2H), 4.52 (t,  $J = 7.3$  Hz, 1H), 3.93 (t,  $J = 6.3$  Hz, 1H), 3.52 (s, 3H), 3.39 (s, 3H), 3.26 (s, 3H), 3.24–3.17 (m, 3H), 3.11 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.31–2.06 (m, 12H), 1.67–1.62 (m, 4H), 1.55–1.21 (m, 12H), 1.07–0.84 (m, 38H); ESI MS  $m/z$  1287 [ $\text{C}_{69}\text{H}_{111}\text{N}_{11}\text{O}_{12} + \text{H}$ ] $^+$ ; HPLC 96.6% (AUC),  $t_R = 20.88$  min.

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### **Example 32 – Preparation of the Acetate of Cyclosporin yne-yne-ene**

[0079] To an ice-cooled solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (25 mg, 0.019 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride (95  $\mu\text{L}$ , 1 M in THF, 0.095 mmol), then the mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then copper(I) iodide (4 mg, 0.019 mmol) and dichlorobis(triphenylphosphine)palladium(II) (13 mg, 0.019 mmol) were added into

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the mixture followed by vinyl iodide (30  $\mu$ L, 0.38 mmol). The resulting reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of cyclosporin yne-yne-ene (6 mg, 25%) as a brown oil:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.43 (d,  $J$  = 9.5 Hz, 1H), 8.07 (d,  $J$  = 6.7 Hz, 1H), 7.82 (d,  $J$  = 8.9 Hz, 1H), 7.64 (d,  $J$  = 7.7 Hz, 1H), 5.80–5.65 (m, 4H), 5.60–5.52 (m, 3H), 5.45–5.33 (m, 2H), 5.28 (dd,  $J$  = 11.9, 3.6 Hz, 1H), 5.17 (t,  $J$  = 7.7 Hz, 1H), 5.02–4.91 (m, 4H), 4.86 (t,  $J$  = 7.4 Hz, 1H), 4.79 (t,  $J$  = 9.4 Hz, 1H), 4.65 (d,  $J$  = 14.0 Hz, 1H), 4.49 (d,  $J$  = 7.0 Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–2.05 (m, 4H), 2.04 (s, 3H), 1.98–1.83 (m, 2H), 1.72–1.60 (m, 3H), 1.45–0.75 (m, 54H); ESI MS  $m/z$  1279 [ $\text{C}_{67}\text{H}_{111}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ .

### **Example 33 – Preparation of Cyclosporin yne-yne-ene**

**[0080]** To a solution of the acetate of cyclosporin yne-yne-ene from Example 32 (14 mg, 0.011 mmol) in MeOH (2 mL) was added potassium carbonate (30 mg, 0.22 mmol), then the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with ethyl acetate (30 mL), then washed with a saturated solution of ammonium chloride (15 mL). The aqueous layer was separated and extracted with ethyl acetate (2  $\times$  20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin yne-yne-ene (10 mg, 72%) as a white solid:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.94 (d,  $J$  = 10.0 Hz, 1H), 7.65 (d,  $J$  = 7.5 Hz, 1H), 7.36 (d,  $J$  = 8.5 Hz, 1H), 7.20 (d,  $J$  = 8.0 Hz, 1H), 5.85–5.67 (m, 3H), 5.57 (dd,  $J$  = 10.5, 3.0 Hz, 1H), 5.46 (d,  $J$  = 7.0 Hz, 1H), 5.29 (dd,  $J$  = 11.5, 4.0 Hz, 1H), 5.12 (d,  $J$  = 10.5 Hz, 1H), 5.08 (t,  $J$  = 7.0 Hz, 1H), 5.05–4.95 (m, 2H), 4.83 (t,  $J$  = 7.0 Hz, 1H), 4.74–4.64 (m, 2H), 4.52 (t,  $J$  = 7.5 Hz, 1H), 3.93 (t,  $J$  = 6.5 Hz, 1H), 3.51 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.11 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.69 (s, 3H), 2.61 (dd,  $J$  = 17.5, 4.0 Hz, 1H), 2.45–2.35 (m, 1H), 2.26 (dd,  $J$  = 17.5, 7.5 Hz, 1H), 2.20–2.07 (m, 5H), 2.03–1.88 (m, 3H), 1.82–1.60 (m, 5H), 1.50–0.80 (m, 53H); ESI MS  $m/z$  1237 [ $\text{C}_{65}\text{H}_{109}\text{N}_{11}\text{O}_{12} + \text{H}$ ] $^+$ ; HPLC >99% (AUC),  $t_R$  = 19.98 min.

**Example 34 – Preparation of the Acetate of *cis*-Cyclosporin yne-yne-ene**

**[0081]** To an ice-cooled solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (53 mg, 0.040 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride (0.20 mL, 1 M in THF, 0.20 mmol), then the mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then copper(I) iodide (4 mg, 0.02 mmol) and dichlorobis(triphenylphosphine)palladium(II) (14 mg, 0.02 mmol) were added into the mixture followed by *cis*-1-bromo-1-propene (68  $\mu$ L, 0.80 mmol). The resulting reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of *cis*-cyclosporin yne-yne-ene (19 mg, 37%) as a pale-brown solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.50 (d,  $J$  = 9.7 Hz, 1H), 8.06 (d,  $J$  = 6.8 Hz, 1H), 7.67 (d,  $J$  = 8.9 Hz, 1H), 7.58 (d,  $J$  = 7.8 Hz, 1H), 6.15–6.03 (m, 1H), 5.70 (dd,  $J$  = 11.0, 3.8 Hz, 1H), 5.58–5.40 (m, 3H), 5.31 (dd,  $J$  = 11.9, 3.6 Hz, 2H), 5.15 (t,  $J$  = 7.2 Hz, 1H), 5.04–4.82 (m, 3H), 4.74 (t,  $J$  = 10.5 Hz, 1H), 4.65 (d,  $J$  = 13.9 Hz, 1H), 4.46 (t,  $J$  = 7.0 Hz, 1H), 3.43 (s, 3H), 3.32 (s, 3H), 3.27 (s, 3H), 3.20 (s, 3H), 3.08 (s, 3H), 2.69 (s, 3H), 2.67 (s, 3H), 2.50–2.09 (m, 6H), 2.05 (s, 3H), 1.98–1.83 (m, 4H), 1.72–1.60 (m, 3H), 1.45–0.75 (m, 58H); ESI MS  $m/z$  1293 [ $\text{C}_{68}\text{H}_{113}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ .

**Example 35 – Preparation of *cis*-Cyclosporin yne-yne-ene**

**[0082]** To a solution of the acetate of *cis*-cyclosporin yne-yne-ene from Example 34 (19 mg, 0.015 mmol) in MeOH (2 mL) was added potassium carbonate (41 mg, 0.30 mmol), then the mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with ethyl acetate (40 mL), then washed with a saturated solution of ammonium chloride (20 mL). The aqueous layer was separated and extracted with ethyl acetate (2  $\times$  30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford *cis*-cyclosporin yne-yne-ene (10 mg, 53%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.97 (d,  $J$  = 9.6 Hz, 1H), 7.70 (d,  $J$  = 7.3 Hz, 1H), 7.42 (d,  $J$  = 8.2 Hz, 1H), 7.25 (overlapped with  $\text{CHCl}_3$ , 1H), 6.15–6.04 (m, 1H), 5.71 (dd,  $J$  = 11.1, 3.9 Hz, 1H), 5.52–5.40 (m, 2H), 5.29 (dd,  $J$  =



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11.5, 3.8 Hz, 1H), 5.15–4.95 (m, 5H), 4.83 (t,  $J = 7.2$  Hz, 1H), 4.76–4.63 (m, 2H), 4.52 (t,  $J = 7.1$  Hz, 1H), 3.91 (t,  $J = 6.4$  Hz, 1H), 3.51 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.61 (dd,  $J = 17.5, 3.8$  Hz, 1H), 2.50–1.85 (m, 7H), 1.80–0.78 (m, 63H); ESI MS  $m/z$  1251 [ $C_{66}H_{111}N_{11}O_{12} + H$ ] $^+$ ; HPLC >99% (AUC),  $t_R = 20.59$  min.

**Example 36 – Preparation of the Acetate of *trans*-Cyclosporin yne-yne-ene**

[0083] To an ice-cooled solution of the acetate of cyclosporin (trimethylsilyl)diyne from Example 24 (60 mg, 0.045 mmol) in triethylamine (1 mL) was added tetrabutylammonium fluoride (0.23 mL, 1 M in THF, 0.23 mmol), then the mixture was stirred for 10 min. The reaction mixture was allowed to warm to room temperature, then copper(I) iodide (4 mg, 0.02 mmol) and dichlorobis(triphenylphosphine)palladium(II) (16 mg, 0.02 mmol) were added into the mixture followed by *trans*-1-bromo-1-propene (77  $\mu$ L, 0.90 mmol). The resulting reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a micro-filter and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the desired acetate of *trans*-cyclosporin yne-yne-ene (26 mg, 45%) as a pale-brown solid:  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  8.45 (d,  $J = 9.3$  Hz, 1H), 8.08 (d,  $J = 6.6$  Hz, 1H), 7.82 (d,  $J = 8.7$  Hz, 1H), 7.64 (d,  $J = 7.8$  Hz, 1H), 6.35–6.18 (m, 1H), 5.70 (dd,  $J = 11.1, 3.9$  Hz, 1H), 5.60–5.35 (m, 5H), 5.28 (dd,  $J = 12.0, 3.3$  Hz, 1H), 5.17 (t,  $J = 6.3$  Hz, 1H), 5.04–4.75 (m, 5H), 4.65 (d,  $J = 13.8$  Hz, 1H), 4.49 (t,  $J = 7.2$  Hz, 1H), 3.43 (s, 3H), 3.30 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.45–2.35 (m, 1H), 2.30–1.85 (m, 10H), 2.03 (s, 3H), 1.80 (dd,  $J = 6.9, 1.5$  Hz, 3H), 1.75–1.58 (m, 3H), 1.45–0.75 (m, 52H); ESI MS  $m/z$  1292 [ $C_{68}H_{113}N_{11}O_{13} + H$ ] $^+$ .

**Example 37 – Preparation of *trans*-Cyclosporin yne-yne-ene**

[0084] To a solution of the acetate of *trans*-cyclosporin yne-yne-ene from Example 36 (25 mg, 0.019 mmol) in MeOH (3 mL) was added potassium carbonate (52 mg, 0.38 mmol), then the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with ethyl acetate (40 mL), then washed with a saturated solution of ammonium chloride (20 mL). The aqueous layer was separated and

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extracted with ethyl acetate (2 × 30 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford the *trans*-cyclosporin yne-yne-ene (15 mg, 63%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.95 (d, *J* = 9.6 Hz, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 6.35–6.20 (m, 1H), 5.71 (dd, *J* = 11.4, 4.2 Hz, 1H), 5.55–5.40 (m, 2H), 5.29 (dd, *J* = 11.4, 4.2 Hz, 1H), 5.18–4.95 (m, 4H), 4.83 (t, *J* = 7.2 Hz, 1H), 4.76–4.63 (m, 2H), 4.52 (t, *J* = 7.2 Hz, 1H), 3.91 (t, *J* = 6.3 Hz, 1H), 3.50 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.12 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.61 (dd, *J* = 17.4, 3.9 Hz, 1H), 2.45–1.85 (m, 7H), 1.80 (dd, *J* = 6.6, 1.5 Hz, 3H), 1.75–0.78 (m, 61H); ESI MS *m/z* 1250 [C<sub>66</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>; HPLC >99% (AUC), *t<sub>R</sub>* = 20.28 min.

### **Example 38 – Preparation of Cyclosporin Alkyne**

**[0085]** A suspension of cyclosporin alkyne from Example 3 (50 mg, 0.04 mmol), cesium carbonate (325 mg, 1.0 mmol), sodium iodide (150 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. Allyl bromide (70 μL, 0.80 mmol) was added dropwise and the resulting mixture was stirred at room temperature overnight. The blue suspension was diluted with Et<sub>2</sub>O and filtered. The filtrate was washed twice with H<sub>2</sub>O. The combined aqueous layers were extracted with Et<sub>2</sub>O. The combined organics were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave cyclosporin alkyne (25 mg, 52%) as an off-white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.13 (d, *J* = 9.5 Hz, 1H), 7.78 (d, *J* = 7.3 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 10.3 Hz, 1H), 5.84–5.75 (m, 1H), 5.69 (dd, *J* = 11.0, 4.2 Hz, 1H), 5.38–4.96 (m, 10H), 4.84 (t, *J* = 7.1 Hz, 1H), 4.74–4.67 (m, 2H), 4.51 (t, *J* = 7.3 Hz, 1H), 3.88 (t, *J* = 6.6 Hz, 1H), 3.49 (s, 3H), 3.37 (s, 3H), 3.27 (s, 3H), 3.14 (s, 3H), 3.09 (s, 3H), 2.93–2.70 (m, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.15–1.99 (m, 7H), 1.90–1.57 (m, 6H), 1.43–1.28 (m, 13H), 1.06–0.82 (m, 40H); ESI MS *m/z* 1227 [C<sub>64</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>; HPLC >99% (AUC), *t<sub>R</sub>* = 19.59 min.

**Example 39 – Preparation of Cyclosporin Alkyne**

[0086] A suspension of cyclosporin alkyne from Example 3 (50 mg, 0.04 mmol), cesium carbonate (326 mg, 1.0 mmol), sodium iodide (152 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. Benzyl bromide (100  $\mu$ L, 0.8 mmol) was added dropwise and the resulting mixture was stirred at room temperature overnight. The blue suspension was diluted with Et<sub>2</sub>O and filtered. The filtrate was washed twice with H<sub>2</sub>O. The combined aqueous layers were extracted with Et<sub>2</sub>O. The combined organics were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave the cyclosporin alkyne (8 mg, 17%) as an off-white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.14 (d, *J* = 9.7 Hz, 1H), 7.78 (d, *J* = 7.3 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 7.35–7.30 (m, 5H), 7.21 (d, *J* = 7.5 Hz, 1H), 5.70 (dd, *J* = 11.0, 4.0 Hz, 1H), 5.38 (d, *J* = 6.8 Hz, 1H), 5.33–5.29 (m, 1H), 5.20–4.96 (m, 5H), 4.83 (t, *J* = 7.3 Hz, 1H), 4.74–4.67 (m, 2H), 4.51 (t, *J* = 7.2 Hz, 1H), 3.88 (t, *J* = 6.5 Hz, 1H), 3.57 (s, 2H), 3.52–3.45 (m, 10H), 3.38 (s, 3H), 3.25 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.16–1.62 (m, 10H), 1.43–1.19 (m, 16H), 1.01–0.79 (m, 35H); ESI MS *m/z* 1277 [C<sub>68</sub>H<sub>113</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>; HPLC >99% (AUC), *t<sub>R</sub>* = 20.43 min.

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**Example 40 – Preparation of 1-(Trimethylsilyl)propyn-3-yl Cyclosporin Alkyne**

[0087] A suspension of cyclosporin alkyne from Example 3 (50 mg, 0.04 mmol), cesium carbonate (326 mg, 1.0 mmol), sodium iodide (152 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. 3-Bromo-1-(trimethylsilyl)-1-propyne (0.11 mL, 0.8 mmol) was added dropwise and the resulting mixture was stirred at room temperature for 15 min. The blue suspension was diluted with Et<sub>2</sub>O and filtered. The filtrate was washed twice with H<sub>2</sub>O. The combined aqueous layers were extracted with Et<sub>2</sub>O. The combined organics were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave the 1-(trimethylsilyl)propyn-3-yl cyclosporin alkyne (26 mg, 50%) as an off-white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.09 (d, *J* = 9.4 Hz, 1H), 7.77 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 5.70 (dd, *J* = 10.9, 3.9 Hz, 1H), 5.38 (d,

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$J = 6.6$  Hz, 1H), 5.28 (dd,  $J = 11.5, 3.6$  Hz, 1H), 5.19–4.96 (m, 4H), 4.84 (t,  $J = 7.4$  Hz, 1H), 4.74–4.66 (m, 2H), 4.51 (t,  $J = 7.2$  Hz, 1H), 3.83 (t,  $J = 6.6$  Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.18 (s, 2H), 3.13 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.15–1.98 (m, 10H), 1.91–1.23 (m, 18H), 1.06–0.83 (m, 41H), 0.15 (s, 9H); ESI MS  $m/z$  1297 [ $C_{67}H_{117}N_{11}O_{12}Si + H$ ] $^{+}$ ; HPLC 96.8% (AUC),  $t_R = 21.59$  min.

**Example 41 – Preparation of Cyclosporin Non-Conjugated Diyne**

10 [0088] To a solution of cyclosporin alkyne from Example 40 (18 mg, 0.01 mmol) in MeOH (1 mL) was added potassium carbonate (20 mg, 0.14 mmol) and then the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with EtOAc, washed with H<sub>2</sub>O (2 ×), brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave the cyclosporin non-  
15 conjugated diyne (8 mg, 43%) as a white solid: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.10 (d,  $J = 9.7$  Hz, 1H), 7.71 (d,  $J = 7.4$  Hz, 1H), 7.46 (d,  $J = 8.3$  Hz, 1H), 7.20 (d,  $J = 7.8$  Hz, 1H), 5.71 (dd,  $J = 10.9, 4.2$  Hz, 1H), 5.40 (d,  $J = 6.6$  Hz, 1H), 5.28 (dd,  $J = 11.6, 3.7$  Hz, 1H), 5.17 (d,  $J = 10.9$  Hz, 1H), 5.09 (t,  $J = 6.7$  Hz, 1H), 5.04–4.98 (m, 2H), 4.84 (app quintet,  $J = 7.2$  Hz, 1H), 4.73–4.66 (m, 2H), 4.52 (app quintet,  $J =$   
20 7.3 Hz, 1H), 3.85 (t,  $J = 6.6$  Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.49–2.38 (m, 2H), 2.17–2.09 (m, 5H), 2.02 (t,  $J = 2.7$  Hz, 1H), 2.01–1.95 (m, 1H), 1.84–1.59 (m, 7H), 1.53–1.41 (m, 4H), 1.36–1.24 (m, 12H), 1.04–0.84 (m, 40H); ESI MS  $m/z$  1225 [ $C_{64}H_{109}N_{11}O_{12} + H$ ] $^{+}$ ; HPLC 99.0% (AUC),  $t_R = 18.61$  min; and cyclosporin alkynylallene (6.7 mg, 36%)  
25 as a white solid: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.07 (d,  $J = 9.6$  Hz, 1H), 7.75 (d,  $J = 7.2$  Hz, 1H), 7.52 (d,  $J = 8.4$  Hz, 1H), 7.29 (d,  $J = 7.8$  Hz, 1H), 5.70 (dd,  $J = 10.9, 2.5$  Hz, 1H), 5.40–5.35 (m, 2H), 5.29 (dd,  $J = 10.9, 4.5$  Hz, 1H), 5.17 (d,  $J = 10.9$  Hz, 1H), 5.10 (t,  $J = 6.4$  Hz, 1H), 5.05–5.01 (m, 2H), 4.96 (d,  $J = 6.9$  Hz, 1H), 4.84 (t,  $J = 7.1$  Hz, 1H), 4.73–4.67 (m, 2H), 4.51 (app quintet,  $J = 7.2$  Hz, 1H), 3.48 (t,  $J =$   
30 6.6 Hz, 1H), 3.38 (s, 3H), 3.26 (s, 3H), 3.14 (s, 3H), 3.10 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.15–2.09 (m, 5H), 2.02–1.61 (m, 7H), 1.55–1.25 (m, 11H), 1.04–0.84 (m, 50H); ESI MS  $m/z$  1225 [ $C_{64}H_{109}N_{11}O_{12} + H$ ] $^{+}$ ; HPLC 91.7% (AUC),  $t_R = 20.45$  min.

**Example 42 – Preparation of the Acetate of Cyclosporin Non-Conjugated Diyne**

**[0089]** A suspension of the acetate of cyclosporin alkyne from Example 6 (50 mg, 0.05 mmol), cesium carbonate (326 mg, 1.0 mmol), sodium iodide (150 mg, 1.0 mmol) and copper(I) iodide (190 mg, 1.0 mmol) in DMF (2 mL) was stirred at room temperature for 30 min. 1-Bromo-2-butyne (90  $\mu$ L, 1.0 mmol) was added dropwise and the resulting mixture was stirred at room temperature overnight. The blue suspension was diluted with Et<sub>2</sub>O and filtered. The filtrate was washed twice with H<sub>2</sub>O. The combined aqueous layers were extracted with Et<sub>2</sub>O. The combined organics were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave the acetate of cyclosporin non-conjugated diyne (47 mg, 73%) as a light brown solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.46 (d, *J* = 9.8 Hz, 1H), 8.10 (d, *J* = 6.8 Hz, 1H), 7.81 (d, *J* = 8.9 Hz, 1H), 7.60 (d, *J* = 7.7 Hz, 1H), 5.70 (dd, *J* = 10.9, 4.0 Hz, 1H), 5.23–5.15 (m, 6H), 5.07–4.62 (m, 5H), 4.87 (t, *J* = 6.9 Hz, 1H), 3.43 (s, 3H), 3.29 (s, 3H), 3.24 (s, 3H), 3.19 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.68 (s, 3H), 2.49–2.09 (m, 13H), 2.05 (s, 3H), 1.74 (t, *J* = 2.5 Hz, 3H), 1.41–1.26 (m, 12H), 1.12–0.82 (m, 44H); ESI MS *m/z* 1281 [C<sub>67</sub>H<sub>113</sub>N<sub>11</sub>O<sub>13</sub> + H]<sup>+</sup>.

**Example 43 – Preparation of Cyclosporin Non-Conjugated Diyne**

**[0090]** To a solution of the acetate of cyclosporin non-conjugated diyne from Example 42 (47 mg, 0.04 mmol) in MeOH (2 mL) was added potassium carbonate (55 mg, 0.4 mmol) and then the mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with H<sub>2</sub>O (2  $\times$ ), brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. Purification by semi-preparative HPLC gave the cyclosporin non-conjugated diyne (23 mg, 46%) as a brown solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.09 (d, *J* = 9.5 Hz, 1H), 7.77 (d, *J* = 7.3 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 7.9 Hz, 1H), 5.70 (dd, *J* = 10.9, 4.0 Hz, 1H), 5.37 (d, *J* = 6.7 Hz, 1H), 5.27 (dd, *J* = 11.5, 3.7 Hz, 1H), 5.20–4.96 (m, 5H), 4.84 (t, *J* = 7.3 Hz, 1H), 4.74–4.65 (m, 2H), 4.51 (t, *J* = 7.2 Hz, 1H), 3.85 (t, *J* = 6.6 Hz, 1H), 3.49 (s, 3H), 3.38 (s, 3H), 3.28 (s, 3H), 3.14 (s, 3H), 3.09 (s, 3H), 2.72 (s, 3H), 2.70 (s, 3H), 2.43–2.01 (m, 15H), 1.78 (t, *J* = 2.5 Hz, 3H), 1.66–1.23 (m, 15H), 1.04–0.83 (m, 40H); ESI MS *m/z* 1239 [C<sub>65</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub> + H]<sup>+</sup>; HPLC >99% (AUC), *t<sub>R</sub>* = 19.45 min.

**Example 44 – Preparation of Cyclosporin Alkynyl Alcohol**

[0091] To a solution of cyclosporin alkyne from Example 3 (100 mg, 0.081 mmol) and paraformaldehyde (133 mg, 0.81 mmol) in DMSO (3 mL) was added benzyltrimethylammonium hydroxide (372  $\mu$ L, 40% solution in methanol, 0.81 mmol) dropwise over 10 min. The resulting solution was stirred at room temperature for 14 h. The reaction was quenched with water and extracted with diethyl ether (4  $\times$  25 mL). The combined organic layers were washed with water, brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by semi-preparative HPLC to afford the cyclosporin alkynyl alcohol (23 mg, 23%) as a white solid:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.95 (d,  $J$  = 9.3 Hz, 1H), 7.52 (d,  $J$  = 7.3 Hz, 1H), 7.46 (d,  $J$  = 8.3 Hz, 1H), 7.20 (d,  $J$  = 7.9 Hz, 1H), 5.69 (dd,  $J$  = 10.8, 4.3 Hz, 1H), 5.45–5.40 (m, 2H), 5.14 (d,  $J$  = 11.0 Hz, 1H), 5.13–5.05 (m, 2H), 5.00–4.95 (m, 2H), 4.83 (t,  $J$  = 6.8 Hz, 1H), 4.64 (dd,  $J$  = 9.8, 8.4 Hz, 1H), 4.52 (t,  $J$  = 7.3 Hz, 1H), 4.03 (d,  $J$  = 6.7 Hz, 2H), 3.94 (t,  $J$  = 6.7 Hz, 1H), 3.51 (s, 3H), 3.31 (s, 3H), 3.27 (s, 3H), 3.13 (s, 3H), 3.09 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.40–0.70 (m, 70H); ESI MS  $m/z$  1217 [ $\text{C}_{62}\text{H}_{109}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ ; HPLC 98.4% (AUC),  $t_R$  = 18.24 min.

**Example 45 – Preparation of Cyclosporin Diol**

[0092] To a mechanically stirred solution of diisopropylamine (2.6 mL, 18 mmol) in THF (50 mL) at  $-78^\circ\text{C}$  was added dropwise *n*-butyllithium (6.6 mL, 2.5 M in hexane, 17 mmol), then the mixture was stirred for 0.5 h. A solution of cyclosporin A (1.0 g, 0.83 mmol) in THF (8 mL) was added, and then the mixture was stirred for 2 h at  $-78^\circ\text{C}$ . Paraformaldehyde (8.0 g) was heated to  $170^\circ\text{C}$  and the resulting formaldehyde gas was transferred into the reaction via a glass tube which was wrapped with cotton and aluminum foil over 2 h. After stirring another 1 h at  $-78^\circ\text{C}$ , the reaction mixture was quenched with water (10 mL). The mixture was allowed to warm to room temperature, diluted with ethyl acetate (150 mL), and washed with water (2  $\times$  50 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diol (0.45 g, 44%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.09 (d,  $J$  = 9.9 Hz, 1H), 7.70 (d,  $J$  = 7.4 Hz, 1H), 7.57 (d,  $J$  = 8.2 Hz, 1H), 7.15 (overlapped with  $\text{CHCl}_3$ , 1H), 5.70 (dd,  $J$

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= 11.0, 4.0 Hz, 1H), 5.49 (d,  $J = 6.4$  Hz, 1H), 5.38–5.30 (m, 3H), 5.16–4.93 (m, 5H), 4.83 (t,  $J = 7.2$  Hz, 1H), 4.65 (t,  $J = 9.5$  Hz, 1H), 4.54 (t,  $J = 7.2$  Hz, 1H), 4.05 (d,  $J = 6.8$  Hz, 2H), 3.73 (t,  $J = 6.3$  Hz, 1H), 3.49 (s, 3H), 3.30 (s, 3H), 3.25 (s, 3H), 3.15 (s, 3H), 3.11 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.50–2.38 (m, 2H), 2.20–1.92 (m, 6H),  
5 1.75–0.65 (m, 64H); ESI MS  $m/z$  1233 [ $C_{63}H_{113}N_{11}O_{13} + H$ ]<sup>+</sup>.

#### **Example 46 – Preparation of Cyclosporin Diacetate**

[0093] To a solution of cyclosporin diol from Example 45 (0.43 g, 0.35 mmol)  
10 in methylene chloride (5 mL) was added pyridine (0.57 mL, 7.0 mmol) followed by 4-(dimethylamino)pyridine (86 mg, 0.70 mmol) and acetic anhydride (1.0 mL, 10.5 mmol). The reaction mixture was stirred for 2 days at room temperature. The reaction was diluted with ethyl ether (150 mL) and washed with a saturated solution of sodium bicarbonate (30 mL), 1N HCl solution (30 mL) and brine (30 mL). The  
15 organic layer was separated, dried over anhydrous sodium sulfate, and concentrated under vacuum. The crude material was purified by semi-preparative HPLC to afford cyclosporin diacetate (0.23 g, 50%) as a white solid: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  
δ 8.60 (d,  $J = 9.8$  Hz, 1H), 8.05 (d,  $J = 6.6$  Hz, 1H), 7.55 (d,  $J = 7.8$  Hz, 1H), 7.49 (d,  $J = 9.3$  Hz, 1H), 5.68 (dd,  $J = 11.0, 4.0$  Hz, 1H), 5.49 (s, 2H), 5.40–4.95 (m, 8H), 4.85  
20 (t,  $J = 7.5$  Hz, 1H), 4.76 (t,  $J = 9.3$  Hz, 1H), 4.58–4.34 (m, 3H), 3.37 (s, 3H), 3.27 (s, 3H), 3.23 (s, 3H), 3.20 (s, 3H), 3.14 (s, 3H), 2.67 (s, 3H), 2.66 (s, 3H), 2.48–2.35 (m, 1H), 2.10 (s, 3H), 2.01 (s, 3H), 1.98–1.85 (m, 2H), 1.75–0.65 (m, 67H); ESI MS  $m/z$   
1317 [ $C_{67}H_{117}N_{11}O_{15} + H$ ]<sup>+</sup>.

#### **Example 47 – Preparation of Cyclosporin Aldehyde**

[0094] Ozone was bubbled into a solution of cyclosporin diacetate from  
Example 46 (0.22 g, 0.17 mmol) in methylene chloride (10 mL) at –78°C until a blue  
color was developed. The mixture was degassed with nitrogen for a few minutes and  
30 dimethylsulfide (0.4 mL) was added at –78°C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate (120 mL), washed with water (2 × 20 mL) and brine (30 mL), dried over sodium sulfate, filtered, and concentrated  
in vacuo to afford cyclosporin aldehyde (0.19 g, 86%) as a white solid. The crude

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material was carried to the next step without further purification:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  9.55 (d,  $J = 3.4$  Hz, 1H), 8.60 (d,  $J = 9.9$  Hz, 1H), 7.96 (d,  $J = 7.1$  Hz, 1H), 7.53 (d,  $J = 7.7$  Hz, 1H), 7.33 (d,  $J = 9.1$  Hz, 1H), 5.68 (dd,  $J = 11.0$ , 4.0 Hz, 1H), 5.53 (d,  $J = 11.2$  Hz, 1H), 5.47 (d,  $J = 11.2$  Hz, 1H), 5.30 (dd,  $J = 12.3$ , 3.6 Hz, 1H), 5.18–4.92 (m, 5H), 4.84 (t,  $J = 6.9$  Hz, 1H), 4.72 (t,  $J = 9.6$  Hz, 1H), 4.55–4.35 (m, 3H), 3.39 (s, 3H), 3.30 (s, 3H), 3.29 (s, 3H), 3.21 (s, 3H), 3.12 (s, 3H), 2.66 (s, 3H), 2.65 (s, 3H), 2.48–2.30 (m, 3H), 2.10 (s, 3H), 1.99 (s, 3H), 1.80–0.75 (m, 64H); ESI MS  $m/z$  1305 [ $\text{C}_{65}\text{H}_{113}\text{N}_{11}\text{O}_{16} + \text{H}$ ] $^+$ .

#### 10 **Example 48 – Preparation of Cyclosporin Alkyne**

[0095] To a solution of cyclosporin aldehyde from Example 47 (715 mg, 0.55 mmol) in methanol (7.5 mL) was added potassium carbonate (760 mg, 5.5 mmol) followed by a solution of dimethyl (1-diazo-2-oxopropyl)phosphonate (1.06 g, 5.5 mmol) in methanol (4.5 mL). The resulting mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure, and then diluted with ethyl acetate (100 mL). The organic layer was washed with water (40 mL). The aqueous layer was extracted with ethyl acetate ( $3 \times 50$  mL). The combined organic layers were dried over anhydrous sodium sulfate, then concentrated under reduced pressure. The crude material was purified by semi-preparative HPLC to yield cyclosporin alkyne (106 mg, 16%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.94 (d,  $J = 9.9$  Hz, 1H), 7.62–7.55 (m, 2H), 7.27 (d,  $J = 9.6$  Hz, 1H), 5.68 (dd,  $J = 11.0$ , 3.8 Hz, 1H), 5.47–5.41 (m, 2H), 5.18–4.92 (m, 6H), 4.91–4.77 (m, 2H), 4.63 (t,  $J = 9.1$  Hz, 1H), 4.52 (t,  $J = 7.1$  Hz, 1H), 4.03 (d,  $J = 6.6$  Hz, 1H), 3.50 (s, 3H), 3.30 (s, 3H), 3.28 (s, 3H), 3.14 (s, 3H), 3.09 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.50–2.21 (m, 3H), 2.20–1.57 (m, 16H), 1.56–0.72 (m, 54H); ESI MS  $m/z$  1217 [ $\text{C}_{62}\text{H}_{109}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ ; HPLC >99% (AUC),  $t_R = 18.20$  min.

#### 30 **Example 49 – Preparation of Cyclosporin yne-ene**

[0096] To a solution of cyclosporin alkyne from Example 48 (43 mg, 0.04 mmol) in triethylamine (1.5 mL) was added copper(I) iodide (4 mg, 0.02 mmol), followed by dichlorobis(triphenylphosphine)palladium(II) (14 mg, 0.02 mmol) and then vinyl iodide (123 mg, 0.8 mmol). The resulting mixture was stirred at room



temperature for 2 h. The solution was filtered through a micro filter and concentrated under reduced pressure. The crude material was purified by semi-preparative HPLC to yield cyclosporin yne-ene (106 mg, 16%) as a white solid:  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.20 (d,  $J = 10.3$  Hz, 1H), 7.75 (d,  $J = 7.0$  Hz, 1H), 7.48 (d,  $J = 8.2$  Hz, 1H), 7.26 (overlapped with  $\text{CHCl}_3$ , 1H), 5.81–5.66 (m, 2H), 5.59–5.50 (m, 1H), 5.46–5.33 (m, 2H), 5.26 (dd,  $J = 11.6, 3.7$  Hz, 1H), 5.20–5.01 (m, 5H), 4.95 (t,  $J = 6.9$  Hz, 1H), 4.84 (t,  $J = 7.6$  Hz, 1H), 4.69 (t,  $J = 9.2$  Hz, 1H), 4.52 (t,  $J = 7.5$  Hz, 1H), 4.03 (d,  $J = 6.5$  Hz, 2H), 3.84 (t,  $J = 6.4$  Hz, 1H), 3.50 (s, 3H), 3.31 (s, 3H), 3.29 (s, 3H), 3.14 (s, 3H), 2.70 (s, 3H), 2.69 (s, 3H), 2.61–2.50 (m, 1H), 2.22–1.54 (m, 16H), 1.53–0.70 (m, 54H); ESI MS  $m/z$  1243 [ $\text{C}_{64}\text{H}_{111}\text{N}_{11}\text{O}_{13} + \text{H}$ ] $^+$ ; HPLC 96.3% (AUC),  $t_R = 21.22$  min.

#### **Example 50 - Concanavalin A- Stimulated Splenocyte Assay**

[0097] Male BALB/c mice, at 5 to 7 weeks of age, were sacrificed by  $\text{CO}_2$  inhalation. Spleens were removed and dissociated by pushing through a nylon cell strainer. The splenocytes were washed in RPMI 1640/5% fetal calf serum (FCS) and pelleted at  $400\times g$ . Red blood cells were then lysed by resuspending the cell pellet in ACK lysis buffer (150 mM  $\text{NH}_4\text{Cl}$ , 1 mM  $\text{KHCO}_3$ , 0.1 mM EDTA, 3 mL per spleen) for 10 min at room temperature. After pelleting at  $400\times g$ , the cells were washed by resuspending in RPMI 1640/5% FCS and repelleting. The cell pellet was resuspended in RPMI 1640/5% FCS and again passed through a cell strainer to remove cell aggregates. The cells were then counted and adjusted to  $2 \times 10^6$  cells/ml in RPMI 1640/10% FCS/50  $\mu\text{M}$  2-mercaptoethanol. Cell viability was assessed by Trypan blue staining. Cyclosporin A or the test compound and two micrograms of concanavalin A were added to the wells of a 96 well plate, prior to the addition of  $2 \times 10^5$  splenocytes. The cells were cultured in a  $37^\circ\text{C}$   $\text{CO}_2$  incubator for 2 days and then pulsed with 1  $\mu\text{Ci}$  of [ $^3\text{H}$ ]thymidine for 6 hours. Cells were harvested onto filtermats with a TomTec 96 well plate harvester and lysed with  $\text{H}_2\text{O}$ . The filtermat and scintillation fluid were sealed in a plastic sleeve. [ $^3\text{H}$ ]thymidine incorporation was measured with a Wallac Trilux plate counter. Initial screens were done at a fixed value of 100 ng/ml test compound.  $\text{IC}_{50}$ s were calculated from 7 point concentration-response curves using graphPad software.

**Example 51 - Murine *Ex Vivo* Pharmacodynamic Assay**

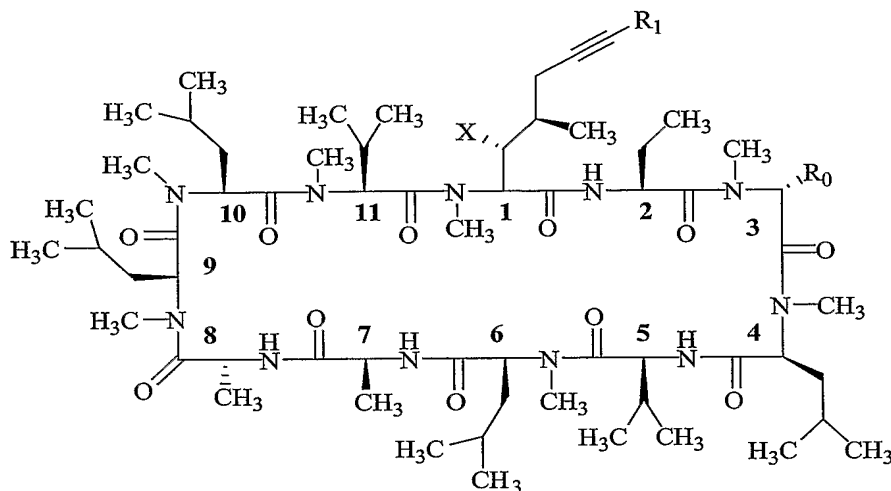
[0098] *In vivo* immunosuppressive activity can be determined for cyclosporin A and the disclosed cyclosporin analog compounds, as described below. The  
5 concanavalin A-stimulated splenocyte activity can be assessed *in vivo* using a method previously described by Peterson et al. (Peterson et al., "A Tacrolimus-Related Immunosuppressant with Biochemical Properties Distinct from Those of Tacrolimus," *Transplantation*, 65:10-18 (1998), which is hereby incorporated by reference in its entirety) or a slightly modified version thereof.

10 [0099] Optimal doses of cyclosporin A or an immunosuppressive compound of the present invention (four different doses of test drug plus a control set of animals with no drug) were administered orally or intravenously to male BALB/c or female C57BL mice. Three mice were tested at each dose. Concanavalin A was injected into the tail vein of the mouse at 4 hours after the administration of cyclosporin A or the  
15 immunosuppressive compound. One hour after the concanavalin A injection, the mice were euthanized, the spleens were removed under sterile conditions, and the extent of splenocyte proliferation was measured in a similar manner, as described in Example 50. The percent inhibition relative to control was plotted graphically versus the dose of the immunosuppressive compound and an ED<sub>50</sub> value was determined.  
20 Each dose-response assay for the compound of the present invention was accompanied by a cyclosporin control at a single dose equal to the ED<sub>50</sub>.

[0100] Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and  
25 scope of the invention which is defined by the following claims.

**WHAT IS CLAIMED IS:**

1. A compound of Formula I:

**Formula I**

wherein:

X is OH or OAc;

R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OR<sub>2</sub>;

R<sub>1</sub> is selected from the group consisting of:

- hydrogen;
- halogen;
- C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain;
- C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain containing substitution or substitutions selected from the group consisting of deuterium, halogen, nitrogen, sulfur, and silicon atom or atoms;
- C<sub>2</sub>-C<sub>6</sub> saturated or unsaturated, straight or branched carbon chain containing a function group or function groups selected from the group consisting of alcohol, ether, aldehyde, ketone, carboxylic ester, and amide;
- C<sub>2</sub>-C<sub>4</sub> saturated or unsaturated, straight or branched carbon chain containing an aryl or a heteroaryl;
- C<sub>3</sub>-C<sub>6</sub>-substituted and unsubstituted cycloalkyl;
- substituted and unsubstituted aryl;
- substituted and unsubstituted heteroaryl;
- CH<sub>2</sub>OH;
- CHO;
- CH=N-OR<sub>3</sub>; and
- CH=N-NR<sub>3</sub>R<sub>4</sub>;

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R<sub>2</sub> is selected from the group consisting of:

alkanoyl;  
alkenoyl;  
alkynoyl;  
aryloyl;  
arylalkanoyl;  
alkylaminocarbonyl;  
arylaminocarbonyl;  
arylalkylaminocarbonyl;  
alkyloxycarbonyl;  
aryloxycarbonyl; and  
arylalkyloxycarbonyl;

R<sub>3</sub> or R<sub>4</sub> are the same or different and independently selected from the group consisting of:

hydrogen;  
C<sub>1</sub>-C<sub>6</sub> saturated straight or branched carbon chain;  
C<sub>3</sub>-C<sub>6</sub> unsaturated straight or branched carbon chain;  
C<sub>3</sub>-C<sub>6</sub>-substituted and unsubstituted cycloalkyl;  
C<sub>1</sub>-C<sub>4</sub> carbon chain containing an aryl or heteroaryl;  
substituted and unsubstituted aryl;  
substituted and unsubstituted heteroaryl;  
alkanoyl;  
alkenoyl;  
alkynoyl;  
aryloyl;  
arylalkanoyl;  
alkylaminocarbonyl;  
arylaminocarbonyl;  
arylalkylaminocarbonyl;  
alkyloxycarbonyl;  
aryloxycarbonyl; and  
arylalkyloxycarbonyl; and

R<sub>3</sub> together with R<sub>4</sub> results in the formation of a cyclic moiety of C<sub>2</sub>-C<sub>6</sub> optionally containing heteroatom or heteroatoms,

or a pharmaceutically acceptable salt thereof.

2. The compound according to claim 1, wherein X is OH or OAc, and R<sub>0</sub> is H, CH<sub>2</sub>OH, or CH<sub>2</sub>OAc.

3. The compound according to claim 2, wherein R<sub>1</sub> is H.

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4. The compound according to claim 2, wherein  $R_1$  is selected from the group consisting of F, Cl, Br, and I.
5. The compound according to claim 2, wherein  $R_1$  is selected from the group consisting of  $CH=CH_2$ ,  $CH=CHCH_3$ ,  $CH=CHCH_2CH_3$ ,  $C(CH_3)=CH_2$ ,  $CH=CD_2$ ,  $CH=CHCD_3$ , and  $CH=CDCD_3$ , and wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.
6. The compound according to claim 2, wherein  $R_1$  is selected from the group consisting of  $CH=CHF$ ,  $CH=CHCl$ ,  $CH=CHBr$ ,  $CH=CHI$ ,  $CH=CF_2$ , and  $CH=CCl_2$ , and wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.
7. The compound according to claim 2, wherein  $R_1$  is selected from the group consisting of  $C\equiv CH$ ,  $C\equiv CCH_3$ ,  $C\equiv CCD_3$ ,  $C\equiv CCH_2CH_3$ ,  $C\equiv CCH_2CH_2CH_3$ , and  $C\equiv C$ -cyclopropyl.
8. The compound according to claim 2, wherein  $R_1$  is selected from the group consisting of  $CH_2C\equiv CH$ ,  $CH_2C\equiv CCH_3$ ,  $CH_2C\equiv CCH_2CH_3$ ,  $CH_2CH=CH_2$ ,  $CH_2CH=CHCH_3$ , and  $CH_2CH=CHCH_2CH_3$ , and wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.
9. The compound according to claim 2, wherein  $R_1$  is selected from the group consisting of  $C\equiv C-C\equiv CH$ ,  $C\equiv C-C\equiv CCH_3$ ,  $C\equiv CCH=CH_2$ ,  $C\equiv CCH=CHCH_3$ ,  $CH=CHC\equiv CH$ ,  $CH=CHC\equiv CCH_3$ ,  $CH=CHCH=CH_2$ , and  $CH=CHCH=CHCH_3$ , and wherein the carbon-carbon double bond is a cis or a trans geometric isomer or a mixture of both cis and trans geometric isomers.
10. The compound according to claim 2, wherein  $R_1$  is cyclopropyl.

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11. The compound according to claim 2, wherein  $R_1$  is selected from the group consisting of  $CH_2OH$ ,  $-CHO$ ,  $CH(OH)CH_3$ ,  $C(=O)CH_3$ ,  $CH=N-OCH_3$ ,  $CH=N-OCH_2CH_3$ ,  $CH=N-NHCH_3$ , and  $CH=N-N(CH_3)_2$ .
12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 1.
13. A method of suppressing or reducing immune response in a mammal comprising:  
administering a therapeutically effective amount of the compound of claim 1 to said mammal under conditions effective to suppress immune response in a mammal.
14. A method of treating a mammal with a chronic inflammatory or autoimmune disease comprising:  
administering a therapeutically effective amount of the compound of claim 1 to the mammal under conditions effective to treat the chronic inflammatory or autoimmune disease.
15. The method of claim 14, wherein the chronic inflammatory or autoimmune disease is selected from the group consisting of asthma, rheumatoid arthritis, multiple sclerosis, psoriasis, and ulcerative colitis.
16. A method of treating a mammal with ocular allergy and dry eye comprising:  
administering a therapeutically effective amount of the compound of claim 1 to the mammal under conditions effective to treat ocular allergy and dry eye.

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**Figure 1**